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(57) Abstract

This concerns modified peptides and their pharmaceutically acceptable salts which can effectively penetrate the blood-brain barrier. Also of concern are pharmaceutical compositions containing these peptides and methods of treatment using such compositions.

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MODIFIED PEPTIDES TRANSPORTABLE INTO THE CENTRAL NERVOUS SYSTEM

Field of the Invention

This invention relates to modified peptides and their pharmaceutically acceptable salts which can effectively penetrate the blood-brain barrier and, in particular, to peptides of no more than six amino acid residues modified by attachment of a lipophilic group to a suitable functional group on the peptide. Also of concern are pharmaceutical compositions containing these peptides and methods of treatment using such compositions.

Background of the Invention

Peptides which are capable of imitating or blocking the effects of a specific biologically active peptide are, in principle, potential therapeutic agents. However, one problem associated with designing such agents is that they are metabolically unstable, i.e., they are degraded by enzymes present within the metabolic pathways of the body through which they must pass. These limitations are discussed in "Design of Metabolically-Stable Peptide Analogs" by Veber et al., TINS, 8: 392-396 (1985).

Neurotensin is an endogenous neurotridecapeptide which is unstable *in vivo*. It exhibits analgesic, antipsychotic, cognition activating, and other effects on the central nervous system (CNS) when injected into the brain or surrounding cerebrospinal fluid. In contrast when neurotensin is administered intravenously, intramuscularly, subcutaneously, or orally it does not exhibit any effects on the CNS because it is

metabolically unstable and is unable to cross the blood-brain barrier.

Overcoming this metabolic instability has presented an interesting challenge to researchers. For example, U.S. Patent 4,425,269, issued to Christy et al. on January 10, 1984 describes metabolically protected linear analogs of the (9-13) fragment of neurotensin having the same activity and substantially the same potency as the tridecapeptide.

European Patent Application Publication 333,071 published on September 20, 1989 describes peptides which are analogs of the C-terminal fragment of neurotensin exhibiting psychotropic activity by virtually any route of administration.

Tsuchiya et al., 200th American Chemical Society National Meeting, Washington, DC August 26-31, 1990, Abstract No. MEDI 15, discloses H-D-Lys^{8}-Arg^{9}-Pro^{10}-Trp^{11}-Tle^{12}-Leu^{13}-OH, a neurotensin (8-13) hexapeptide analog as a subcutaneously active psychotropic substance.

Other peptides which researchers have attempted to modify include the following:

U.S. Patent 3,705,141, issued to Krimmel on December 5, 1972, describes amino acids protected at the N-terminal end by adamantane- and homoadamantanecarbonyl groups. These compounds are described as having antibiotic utility.

European Patent Application Publication 296,892 published on December 28, 1988 discloses N-terminal protected peptides which are antagonists of the antidiuretic and/or vasopressor activity of arginine vasopressin, in which 1-adamantaneacetyl is a protecting group.

U.S. Patent 4,273,704, issued to Mazur on June 16, 1981, describes N-terminal adamantane-substituted

tetrapeptide amides which are enkephalin analogs wherein methionine or leucine at position 5 has been replaced by the adamantyl amide. Analgesic activity, as measured by the mouse PQW test for the lead compound, is reported as ED_{50} 0.31 mg/kg (sc, 60 min).

In addition to metabolic instability, penetration of the blood-brain barrier is another hurdle which researchers face in making neurologically active peptides. The blood-brain barrier severely limits the bioavailability of such peptides to the nervous system. The nature of the blood-brain barrier and problems associated with transport of peptides and proteins therethrough is set forth in Pardridge, Endocrine Reviews, 7(3): 314-330 (August 1986).

The blood-brain barrier serves as a highly important protective device for the extremely sensitive neural tissue. This barrier acts as a system-wide cellular membrane which separates the brain interstitial space from the blood.

The unique morphologic characteristics of the brain capillaries which constitute this barrier are the following: (a) epithelial-like high resistance tight junctions which literally cement all endothelia of brain capillaries together, and (b) scanty pinocytosis or transendothelial channels, which are abundant in endothelia of peripheral organs.

Due to these unique characteristics, compounds which readily gain access to other tissues in the body are barred from entry into the brain or the rates of entry into the brain are extremely low.

Strategies for peptide delivery through the blood-brain barrier which are discussed in Pardridge above include invasive procedures, pharmacologically based strategies, and physiologically based strategies.

One pharmacologically based approach is peptide latentiation or conversion of water-soluble functional groups on the peptide to lipid-soluble derivatives. One form of latentiation of dipeptides is formation of the cyclized derivative, or diketopiperazine. The coupling of the terminal carboxy and amino groups to form the diketopiperazine results in log order increases in the lipid solubility of the compound owing to the loss of several hydrogen bond-forming functional groups on the parent molecule.

Mazurov et al., Khim.-Farm. Zhurnal., 23(7): 812-816 (1989) describe adamantylhydrazide derivatives of thyrotropin releasing hormone (TRH, thyroliberin). Although the emphasis of the study was to increase the spectrum of biological activity of TRH, it is stated on page 812 that introduction of non-naturally occurring units into naturally-occurring peptides appeared to increase resistance to enzymatic degradation. There is mention of transport across the blood-brain barrier but no data is provided to support this proposition.

U.S. Patent No. 4,933,324, issued to Shashoua on June 12, 1990, describes the formation of a prodrug from a fatty acid carrier and a neuroactive drug. The prodrug is described as being preferably inactive until such time as it is hydrolyzed. Once in the central nervous system, the prodrug is hydrolyzed into the fatty acid carrier and the drug.

U.S. Patent No. 4,514,332, issued to Hansen, Jr., et al. on April 30, 1985, describes tetrapeptide adamantyl derivatives useful in the treatment of hypertension.

Summary of the Invention

This invention concerns a compound of the formula

$$Y-(W)_m-(X)_n-A'-H-A-B-C-D-E-F-Z$$

wherein

Y is a lipophilic moiety having the structure L-C(O)-, or $R-(CH_2)_p-C(O)-(CH_2)_r-$, provided that when Y is L-C(O)- then L is selected from the group consisting of (i) at least one alkyl group having 1-16 carbon atoms, said alkyl group can be branched or unbranched, unsubstituted or substituted with at least one cyclic moiety selected from the group consisting of a cycloalkyl group having 3-8 carbon atoms, a heterocyclic group having 5-7 atoms in which the heteroatom is N, O, or S, or an aryl group having 6-15 carbon atoms wherein said aryl group can be unsubstituted or substituted with at least one alkyl group having 1-4 carbon atoms, (ii) perfluoroalkyl having 1-10 carbon atoms which can be unsubstituted or substituted with at least one cyclic group selected from the group consisting of an aryl group having 6-10 carbon atoms, a cycloalkyl group having 3-8 carbon atoms, or a heterocyclic group having 5-7 atoms in which the heteroatom is N, O, or S, (iii) cycloalkyl having 3-8 carbon atoms, (iv) bicycloalkyl having 6-18 carbon atoms, (v) tricycloalkyl having 6-18 carbon atoms, (vi) R^1-NH-R^2 wherein R^1 is H or alkyl having 1-4 carbon atoms; R^2 is selected from the group consisting of alkanediyl, branched or unbranched, having 1-16 carbon atoms, unsubstituted or substituted with at least one cyclic group selected from the group consisting of cycloalkyl having 3-8 carbon atoms, heterocyclic having 5-7 atoms in which the heteroatom is N, O, or S, or an aryl group having 6-15 carbon atoms unsubstituted or substituted with at least one alkyl

group having 1-4 carbon atoms, alkylcycloalkyl branched or unbranched having 4-16 carbon atoms wherein the cycloalkyl group has 3-8 carbon atoms, cycloalkylalkyl branched or unbranched having 4-16 carbon atoms wherein the cycloalkyl group has 3-8 carbon atoms, alkylaryl substituted with at least one moiety selected from the group consisting of alkyl, branched or unbranched, having 7-16 carbon atoms, said alkyl group being unsubstituted or substituted with NHR^1 or OH, said aryl group being unsubstituted or substituted with at least one alkyl group having 1-4 carbon atoms, arylalkyl substituted with at least one moiety selected from the group consisting of alkyl, branched or unbranched, having 7-16 carbon atoms, said alkyl group being unsubstituted or substituted with NHR^1 or OH, said aryl group being unsubstituted or substituted with at least one alkyl group having 1-4 carbon atoms, or alkylheterocyclic substituted with an alkyl group, branched or unbranched, having 6-16 carbon atoms, said heterocyclic having 5-7 atoms in which the heteroatom is N, O, or S,

further provided that when Y is $R-(CH_2)_p-C(O)-(CH_2)_r-$ then R is a cyclic group selected from the group consisting of cycloalkyl having 3-8 carbon atoms, heterocyclic having 5-7 atoms in which the heteroatom is N, O, S, or heterocyclic having 5-7 atoms in which the heteroatom is N and said heterocycle has at least one carbonyl moiety adjacent to the heteroatom, or aryl having 6-15 carbon atoms unsubstituted or substituted with at least one alkyl group having 1-4 carbon atoms; p and r are independently integers from 0 to 6;

W is an amino acid residue selected from the group consisting of arginine, lysine, ornithine, homoarginine, 2,4-diaminobutyric acid, 2,3-diaminopropionic acid,

norleucine, N-methylnorleucine, D-arginine, D-lysine, proline, and 4-aminocyclohexylalanine;

X is an amino acid residue selected from the group consisting of arginine, lysine, ornithine, homoarginine, 2,4-diaminobutyric acid, 2,3-diaminopropionic acid, norleucine, N-methylnorleucine, D-arginine, D-lysine, proline, 4-aminocyclohexylalanine, alanine, or an alpha-amino acid residue substituted at the alpha carbon with at least one alkyl group having 1-6 carbon atoms, or said alpha-carbon atom is part of a cyclic moiety selected from the group consisting of cycloalkyl having 3-8 carbon atoms or heterocyclic having 3-8 atoms in which the heteroatom is N, O, or S;

m and n are independently 0 or 1, provided that m and n are not both 0 unless L is R^1-NH-R^2;

A', A, C, and E are independently selected from the group consisting of -CONH-, -CON(CH_3)-, -N(CH_3)CO-, -NHCR'R"-, -CR'R"NH-, -SO_2NR'R"-, -NR'R"SO_2-, -CH_2NH-, -CH_2O-, -CH_2S-, -NHCH_2-, -OCH_2-, -CSNH-, -NHCONH-, -S(O)CH_2-, -S(O)$_2$$CH_2$-, -NHSC-, -$CH_2$S(O)-, -$CH_2$S(O)$_2$-, -S$CH_2$-, cis- or trans- -CH=CH-, -NHCO-, -CH_2CH_2-, -CF_2CF_2-, -CF=CF-, -CF=CH-, -CH=CF-, -COCH_2-, -CH_2CO-, -CH(OH)CH_2-, -CH_2CH(OH)-, 1,2-cyclopropyldiyl, and 4,5-tetrazolyldiyl, wherein R' and R'' are independently lower alkyl groups having 1-6 carbon atoms;

H is an amino acid residue selected from the group consisting of proline or N-methylaminobutyric acid;

B is an amino acid residue selected from the group consisting of tyrosine, phenylalanine, tryptophan, naphthylalanine, phenylglycine, and beta-phenylproline;

D is an amino acid residue selected from the group consisting of isoleucine, leucine, tert-leucine, and phenylglycine;

F is an amino acid residue selected from the group consisting of leucine, valine, and methionine; and

Z is OH or OR^3 wherein R^3 is an alkyl group having 1-6 carbon atoms.

A preferred embodiment of the invention is one wherein Y is a lipophilic moiety having the structure L-C(O)- or $R-(CH_2)_p-C(O)-(CH_2)_r-$, provided that when Y is L-C(O)- then L is selected from the group consisting of (i) alkyl, branched or unbranched, having 1-16 carbon atoms, (ii) perfluoroalkyl having 1-10 carbon atoms, (iii) cycloalkyl having 3-8 carbon atoms, (iv) bicycloalkyl having 6-18 carbon atoms, (v) tricycloalkyl having 6-18 carbon atoms, (vi) R^1-NH-R^2- wherein R^1 is H or alkyl having 1-4 carbon atoms, R^2 is selected from the group consisting of alkanediyl, branched or unbranched having 1-16 carbon atoms, alkylaryl substituted with at least one moiety selected from the group consisting of alkyl, branched or unbranched, having 7-16 carbon atoms, said alkyl group being unsubstituted or substituted with NHR^1 or OH, said aryl group being unsubstituted or substituted with at least alkyl group having 1-4 carbon atoms, or arylalkyl substituted with at least one moiety selected from the group consisting of alkyl, branched or unbranched, having 7-16 carbon atoms, said alkyl group being unsubstituted or substituted with NHR^1 or OH, said aryl group being unsubstituted or substituted with at least one alkyl group having 1-4 carbon atoms;

further provided that when Y is $R-(CH_2)_p-C(O)-(CH_2)_r$ then R is a cyclic group selected from the group consisting of cycloalkyl having 3-8 carbon atoms, aryl having 6-15 carbon atoms unsubstituted or substituted with at least one alkyl group having 1-4 carbon atoms, heterocyclic having 5-7 atoms in which the heteroatom is N, O, or S, or heterocyclic having 5-7 atoms in which the heteroatom is N and said heterocycle has at least

one carbonyl moiety adjacent to the heteroatom; p and r are independently integers from 0 to 6;

W is an amino acid residue selected from the group consisting of arginine, lysine, ornithine, 2,4-diaminobutyric acid, norleucine, N-methylnorleucine, D-arginine, 4-aminocyclohexylalanine, or proline;

X is an amino acid residue selected from the group consisting of arginine, lysine, ornithine, 2,4-diaminobutyric acid, norleucine, N-methylnorleucine, D-arginine, proline, 4-aminocyclohexylalanine, alanine, or an alpha-amino acid residue in which the alpha carbon is part of cyclic moiety selected from the group consisting of cycloalkyl having 3-8 carbon atoms or heterocyclic having 3-8 atoms in which the hetero atom is N, O, or S;

m and n are independently 0 or 1, provided that m and n are not both 0 unless L is R^1-NH-R^2-;

A', A, C, and E are independently selected from the group consisting of -CONH-, -CH_2NH-, -CH_2O-, -CH_2S-, -NHCH_2-, -OCH_2-, -CSNH-, -NHSC-, -SCH_2-, cis- or trans- -CH=CH-, -NHCO-, -CH_2CH_2-, -CF_2CF_2-, -CF=CF-, -CF=CH-, -CH=CF-, -$COCH_2$-, -CH_2CO-, -CH(OH)CH_2-, -CH_2CH(OH)-;

H is an amino acid residue selected from the group consisting of proline or N-methylaminobutyric acid;

B is an amino acid residue selected from the group consisting of tyrosine, phenylalanine, tryptophan, naphthylalanine, phenylglycine, and beta-phenylproline;

D is an amino acid residue selected from the group consisting of isoleucine, leucine, tert-leucine, and phenylglycine;

F is an amino acid residue selected from the group consisting of leucine, valine, and methionine; and

Z is OH or OR^3 wherein R^3 is alkyl having 1-6 carbon atoms.

A more preferred embodiment is one wherein

Y is selected from the group consisting of acetyl, pivaloyl, neopentylcarbonyl, n-perfluoroctanoyl, 1-bicyclo[3.3.0]octanecarbonyl, 2-bicyclo[2.2.1]heptane-acetyl, 1-adamantanecarbonyl, 2-pyrrolidinecarbonyl (prolyl), 2-(5-pyrrolid-5-one)-carbonyl[pyroglutamyl], benzoyl, 4-tert-butylbenzoyl, 4-phenylbenzoyl, nicotinoyl, 2-benzyl-5-aminopentanoyl, trans-4-(aminomethyl)-cyclohexanecarbonyl, 2-(aminomethyl)-benzoyl, and 4-(aminocyclohexyl)-alanyl;

W is an arginine residue;

X is an amino acid residue selected from the group consisting of arginine, lysine, ornithine, 4-aminocyclohexylalanine, 4-aminopiperidine-4-carboxylic acid, 1-aminocyclopentanecarboxylic acid, 1-aminocyclobutanecarboxylic acid, or 1-amino-cyclopropanecarboxylic acid;

m and n are independently 0 or 1, provided that m and n are not both zero, except when Y is 2-benzyl-5-aminopentanoyl then m and n can be zero, and further provided that when Y is acetyl then m and n are 1;

A', C, and E are -CONH-;

A is -CONH- or $-CH_2NH-$;

H is a proline residue;

B is an amino acid residue selected from the group consisting of tyrosine and tryptophan;

D is an amino acid residue selected from the group consisting of isoleucine, tert-leucine, and phenylglycine;

F is a leucine residue;

Z is OH or OCH_3.

The most preferred embodiments of the invention are those wherein

Y is selected from the group consisting of 1-adamantanecarbonyl, 2-benzyl-5-aminopentanoyl, benzoyl, nicotinoyl, and acetyl;

W is an arginine residue;

X is an amino acid residue selected from the group consisting of arginine, lysine, and ornithine;

m and n are independently 0 or 1, provided that m and n are not both zero, except when Y is 1-benzyl-5-aminopentanoyl then m and n can be zero, and further provided that when Y is acetyl, both m and n are 1;

A', A, C, and E are -CONH-;

H is a proline residue;

B is an amino acid residue selected from the group consisting of tyrosine and tryptophan;

D is an amino acid residue selected from the group consisting of isoleucine, tert-leucine, and phenylglycine;

F is a leucine residue;

Z is OH or OCH_3.

Specific embodiments of the invention are the chemical compounds shown in the following Table according to Example number. All amino acids are the natural optical isomer, L, unless otherwise noted. All peptides exist as the carboxylic acids unless otherwise noted. In addition to the usual amino acid abbreviations, the following abbreviations are used as set forth herein.

N^{α}-alkyl amino acids are represented by the following abbreviations:

(Me)Nle = N^{α}-methylnorleucine

Ada = 1-adamantanecarbonyl

Ala = alanine

Arg = arginine

Boc = t-butoxycarbonyl

Cbz = benzyloxycarbonyl
Cha(4-NH_2) = 4-aminocyclohexylalanine
Fmoc = fluorenyl-9-methoxycarbonyl
Ile = isoleucine
Lys = lysine
Nle = norleucine
Orn = ornithine
Pgl = 2-phenylglycine
pGlu = pyroglutamic acid
Pro = Proline
Tle = 2-t-butylglycine (tert-leucine)
Trp = tryptophan
Tyr = tyrosine
$\Psi[CH_2NH]$ = reduced amide peptide bond isostere
$\Psi[CH{=}CH]$ = trans alkene peptide bond isostere

Table 1

Ex. No.	Chemical Designation	SEQ ID NO
35	N-(2-benzyl-5-aminopentanoyl)-Pro-Tyr-Ile-Leu	88
3	N^{α}-(1-adamantanecarbonyl)-Lys-Pro-Tyr-Ile-Leu	3
8	N^{α}-(1-adamantanecarbonyl)-Orn-Pro-Tyr-Ile-Leu	6
16	N^{α}-benzoyl-Lys-Pro-Tyr-Ile-Leu	50
13	N^{α}-nicotinoyl-Lys-Pro-Tyr-Ile-Leu	43
25	N^{α}-(1-adamantanecarbonyl)-Arg-Arg-Pro-Tyr-Tle-Leu	70
25	N^{α}-acetyl-Arg-Arg-Pro-Tyr-Pgl-Leu	38
25	N^{α}-(1-adamantanecarbonyl)-Lys-Pro-Tyr-Tle-Leu	75
12	N^{α}-(1-adamantanecarbonyl)-Lys-Pro-Tyr-Ile-Leu, methyl ester	41
9	N^{α}-(1-adamantanecarbonyl)-Lys-Pro-Trp-Ile-Leu	7

Detailed Description of the Invention

The term "amino acid" as used herein means an organic compound containing both a basic amino group and

an acidic carboxyl group. Included within this term are modified and unusual amino acids as well as amino acids which are known to occur biologically in free or combined form but usually do not occur in proteins.

The term "amino acid residue" as used herein means that portion of an amino acid (as defined herein) that is present in a peptide/pseudopeptide.

The term "peptide" as used herein means a linear compound that consists of two or more α-amino acids (as defined herein) that are linked by means of peptide or pseudopeptide bonds. Thus, the term "peptide" refers to both peptides and pseudopeptides. A pseudopeptide is a compound which mimics the appearance of a peptide either by using linking groups other than amide linkages between the residual units and/or by using unnatural amino acids as described above and/or an amino acid residue modified in such a way as to render it stable to enzymatic hydrolysis and make it bioavailable.

The term "peptide bond" means a covalent link formed by splitting out a molecule of water between the carboxyl group of one amino acid and the amino group of a second amino acid. This term includes "pseudopeptide bonds" or peptide bond isosteres which are substitutes for the normal amide linkage. These substitute linkages are formed from combinations of atoms not normally found in peptides or proteins which mimic the spatial requirements of the amide bond and which should stabilize the molecule to enzymatic degradation.

It has been found that peptides consisting of up to six amino acid residues can be effectively transported into the central nervous system from the circulatory system by attaching a lipophilic group to a suitable position of the peptide, e.g., to a functional group such as amino, carboxylic acid, or hydroxyl or directly to an appropriate carbon atom. This approach allows

substances, hitherto inaccessible to the brain because of their inability to cross the blood-brain barrier, to become effective pharmaceutical agents useful for treating afflictions of the central nervous system, such as pain, loss of cognitive function, and schizophrenia, etc. Furthermore, such lipophilic groups may protect susceptible peptide bonds from enzymatic cleavage by virtue of their size.

In addition to penetration of the blood-brain barrier, compounds of the invention can be stabilized against enzyme degradation by incorporating: (1) non-hydrolyzable equivalents of the amide bond present as the linking unit between amino acid residues, and/or (2) by using modified or unusual amino acids which are not susceptible to enzymatic degradation.

Examples of non-hydrolyzable equivalents of the amide bond include, but are not limited to, the following: $-CON(CH_3)-$, $-N(CH_3)CO-$, $-NHCR'R"-$, $-CR'CR"NH-$, $-SO_2NR'R"-$, $-NR'R"SO_2-$, $-CH_2NH-$, $-CH_2O-$, $-CH_2S-$, $-NHCH_2-$, $-OCH_2-$, $-CSNH-$, $-NHCONH-$, $-S(O)CH_2-$, $-S(O)_2CH_2-$, $-NHSC-$, $-CH_2S(O)-$, $-CH_2S(O)_2-$, $-SCH_2-$, cis- or trans- $-CH=CH-$, $-CH_2CH_2-$, $-CF_2CF_2-$, $-CF=CF-$, $-CF=CH-$, $-CH=CF-$, $-COCH_2-$, $-CH_2CO-$, $-CH(OH)CH_2-$, $-CH_2CH(OH)-$, 1,2-cyclopropyldiyl, and 4,5-tetrazolyldiyl wherein R' and R" are independently lower alkyl having 1-6 carbon atoms. Non-hydrolyzable equivalents such as monofluorovinyl linkers can be incorporated into peptides using a process such as that described by Allmendinger et al. in Tetrahedron Letters, 31: 7297-7300 (1990).

Modified or unusual amino acids which can be used to practice the invention include, but are not limited to, hydroxylysine, 4-hydroxyproline, ornithine, 2,4-diaminobutyric acid, homoarginine, norleucine, N-methylaminobutyric acid, naphthylalanine, phenylglycine,

ß-phenylproline, tert-leucine, D-arginine, 4-aminocyclohexylalanine, N-methyl-norleucine, D-lysine, 3,4-dehydroproline, 4-aminopiperidine-4-carboxylic acid, 6-aminocaproic acid, trans-4-(aminomethyl)-cyclohexanecarboxylic acid, 2-, 3-, and 4-(aminomethyl)-benzoic acid, 1-aminocyclopentanecarboxylic acid, 1-aminocyclopropanecarboxylic acid, and 2-benzyl-5-aminopentanoic acid.

The compounds of the invention are made as described below. Biological evaluations involve a combination of in vitro and in vivo assays as described below.

One embodiment of this invention concerns compounds which are analogs of neurotensin, in particular, analogs of the C-terminal fragment of neurotensin, for which the amino acid sequences (positions 8-13) and (positions 9-13) are known to possess substantially all the biological activity of the intact natural tridecapeptide.

Introduction of these two peptides into the brain by intracerebroventricular administration produces profound analgesia of limited duration in laboratory animals such as mice and rats. These peptides are substantially devoid of this activity when administered by oral, subcutaneous, or intravenous routes. This inactivity indicates lack of bioavailability except by direct application to the site of action.

It has been found that when the α-amino group of the N-terminal amino acid of NT (8-13) (arginine) and NT (9-13) (arginine) or analogs having other N-terminal amino acids such as lysine, ornithine, etc., are protected by attaching a lipophilic group, such as 1-adamantanecarbonyl, these peptides become more lipophilic and, then, exhibit analgesic effects both by intracerebroventricular and intravenous routes of

administration. Thus, NT (9-13) is inactive when administered intravenously. In contrast, a 1-adamantanecarbonyl derivative is active and has an ED50 value of 2.2 mg/kg. Additionally, the protected α-amino group of the N-terminal acid can be replaced entirely by a lipophilic moiety, such as a benzyl group, to produce a derivative having substantially the same intravenous analgesic activity.

Furthermore, the duration of the analgesic effects is increased using the neurotensin analog. Indeed, the duration exceeds 160 minutes when administered intravenously or intracerebroventricularly whereas the natural peptide exhibits such effects for only a few minutes when administered by the intracerebro-ventricular route.

Neurotensin analogs of the invention can also be used as antipsychotic agents to treat diseases such as schizophrenia. Because neurotensin modulates dopaminergic neurons without blockade of the receptor system, especially in the nigrostriatal region, neurotensin has antipsychotic properties and is devoid of the adverse motor effects known as extrapyramidal symptoms which are characteristic of typical neuroleptics such as chlorpromazine and haloperidol.

Pharmaceutically acceptable salts of the compounds of the invention can be prepared by reacting the free acid or base forms of these peptides with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists of suitable salts are found in A. R. Gennaro, ed., Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, PA, 1985, p. 1418,

the disclosure of which is hereby incorporated by reference.

Compounds of the invention can be synthesized from their carboxy terminal end to their amino terminal end using standard synthetic methods known to those skilled in the art. Generally, peptides are elongated by deprotecting the α-amine of the C-terminal residue and coupling the next suitably protected amino acid through a peptide linkage using the methods described. This deprotection and coupling procedure is repeated until the desired sequence is obtained. This coupling can be performed with the constituent amino acids in a stepwise fashion, or by condensation of fragments (two to several amino acids), or combination of both processes, or by solid phase peptide synthesis according to the method originally described by Merrifield, J. Am. Chem. Soc., 1963, 85, 2149-2154, the disclosure of which is hereby incorporated by reference.

Alternatively, compounds of the invention can be synthesized using automated peptide synthesizing equipment. In addition to the foregoing, peptide syntheses are described in Stewart and Young, "Solid Phase Peptide Synthesis", 2nd ed., Pierce Chemical Co., Rockford, IL (1984); Gross, Meienhofer, Udenfriend, Eds., "The Peptides: Analysis, Synthesis, Biology", Vol 1, 2, 3, 5, and 9, Academic Press, New York, 1980-1987; Bodanszky, "Peptide Chemistry: A Practical Textbook", Springer-Verlag, New York (1988); and Bodanszky, et al. "The Practice of Peptide Synthesis" Springer-Verlag, New York (1984), the disclosures of which are hereby incorporated by reference.

Coupling between two amino acids, an amino acid and a peptide, or two peptide fragments can be carried out using standard coupling procedures such as the azide method, mixed carbonic acid anhydride (isobutyl

chloroformate) method, carbodiimide (dicyclohexylcarbodiimide, diisopropylcarbodiimide, or water-soluble carbodiimide) method, active ester (p-nitrophenyl ester, N-hydroxysuccinic imido ester) method, Woodward reagent K method, carbonyldiimidazole method, phosphorus reagents such as BOP-Cl, or oxidation-reduction method. Some of these methods (especially the carbodiimide method) can be enhanced by adding 1-hydroxybenzotriazole. These coupling reactions can be performed in either solution (liquid phase) or solid phase.

The functional groups of the constituent amino acids must be protected during the coupling reactions to avoid formation of undesired bonds. The protecting groups that can be used are listed in Greene, "Protective Groups in Organic Synthesis", John Wiley & Sons, New York (1981) and "The Peptides: Analysis, Synthesis, Biology", Vol. 3, Academic Press, New York (1981), the disclosure of which is hereby incorporated by reference.

The α-carboxyl group of the C-terminal residue is usually protected by an ester that can be cleaved to give the carboxylic acid. Protecting groups which can be used include: 1) alkyl esters such as methyl and t-butyl, 2) aryl esters such as benzyl and substituted benzyl, or 3) esters which can be cleaved by mild base treatment or mild reductive means such as trichloroethyl and phenacyl esters. When a solid phase synthetic approach is employed, the C-terminal amino acid is attached to an insoluble carrier (usually polystyrene). These insoluble carriers contain a group which will react with the carboxyl group to form a bond which is stable to the elongation conditions but readily cleaved later. Examples of which are: chloro- or bromomethyl resin, hydroxymethyl resin, and aminomethyl resin. Many

of these resins are commercially available with the desired C-terminal amino acid already incorporated.

The α-amino group of each amino acid must be protected. Any protecting group known in the art can be used. Examples of which include: 1) acyl types such as formyl, trifluoroacetyl, phthalyl, and p-toluene-sulfonyl; 2) aromatic carbamate types such as benzyloxycarbonyl (Cbz or Z) and substituted benzyloxycarbonyls, 1-(p-biphenyl)-1-methylethoxy-carbonyl, and 9-fluorenylmethyloxycarbonyl (Fmoc); 3) aliphatic carbamate types such as tert-butyloxycarbonyl (Boc), ethoxycarbonyl, diisopropyl-methoxycarbonyl, and allyloxycarbonyl; 4) cyclic alkyl carbamate types such as cyclopentyloxycarbonyl and adamantyloxycarbonyl; 5) alkyl types such as triphenylmethyl and benzyl; 6) trialkylsilane such as trimethylsilane; and 7) thiol containing types such as phenylthiocarbonyl and dithiasuccinoyl. The preferred α-amino protecting group is either Boc or Fmoc. Many amino acid derivatives suitably protected for peptide synthesis are commercially available.

The α-amino protecting group is cleaved prior to the coupling of the next amino acid. When the Boc group is used, the methods of choice are trifluoroacetic acid, neat or in dichloromethane, or HCl in dioxane. The resulting ammonium salt is then neutralized either prior to the coupling or <u>in situ</u> with basic solutions such as aqueous buffers, or tertiary amines in dichloromethane or dimethylformamide. When the Fmoc group is used, the reagents of choice are piperidine or substituted piperidines in dimethylformamide, but any secondary amine or aqueous basic solutions can be used. The deprotection is carried out at a temperature between 0°C and room temperature.

Any of the amino acids bearing side chain functionalities must be protected during the preparation of the peptide using any of the above-described groups. Those skilled in the art will appreciate that the selection and use of appropriate protecting groups for these side chain functionalities depends upon the amino acid and presence of other protecting groups in the peptide. The selection of such a protecting group is important in that it must not be removed during the deprotection and coupling of the α-amino group.

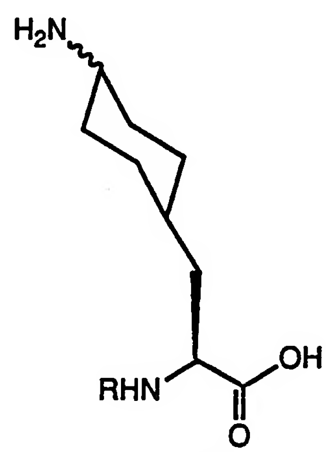
For example, when Boc is chosen for the α-amine protection the following protecting groups are acceptable: p-toluenesulfonyl (tosyl) moieties and nitro for arginine; benzyloxycarbonyl, substituted benzyloxycarbonyls, or tosyl for lysine; benzyl or alkyl esters such as cyclohexyl for glutamic and aspartic acids; benzyl ethers for serine and threonine; benzyl ethers, substituted benzyl ethers or 2-bromobenzyloxycarbonyl for tyrosine; p-methylbenzyl, p-methoxybenzyl, acetamidomethyl, benzyl, or t-butylsulfonyl for cysteine; and the indole of tryptophan can either be left unprotected or protected with a formyl group.

When Fmoc is chosen for the α-amine protection usually tert-butyl based protecting groups are acceptable. For instance, Boc can be used for lysine, tert-butyl ether for serine, threonine and tyrosine, and tert-butyl ester for glutamic and aspartic acids.

Once the elongation of the peptide is completed all of the protecting groups are removed. When a liquid phase synthesis is used, the protecting groups are removed in whatever manner as dictated by the choice of protecting groups. These procedures are well known to those skilled in the art.

When a solid phase synthesis is used, the peptide is cleaved from the resin usually simultaneously with the protecting group removal. When the Boc protection scheme is used in the synthesis, treatment with anhydrous HF containing additives such as dimethyl sulfide, anisole, thioanisole, or p-cresol at 0°C is the preferred method for cleaving the peptide from the resin. The cleavage of the peptide can also be accomplished by other acid reagents such as trifluoromethanesulfonic acid/trifluoroacetic acid mixtures. If the Fmoc protection scheme is used the N-terminal Fmoc group is cleaved with reagents described earlier. The other protecting groups and the peptide are cleaved from the resin using solutions of trifluoroacetic acid and various additives such as anisole, etc.

4-Aminocyclohexylalanine (Cha(4-NH_2)) was synthesized using Boc-p-nitrophenylalanine as described by Nutt et al., "Peptides: Structure and Function"; Proceedings of the Ninth American Peptide Symposium, p. 441 (1985).



Cha(4-NH_2).

Compounds of general formula (I) can be prepared by standard solution phase peptide synthesis. Scheme I outlines the coupling process wherein Compound (IV), an unprotected amine peptide residue is allowed to react with the mixed anhydride form, Compound (V), of the N-protected amino acid residue. Compound (V) is prepared by treatment of the acid (VI) with isobutyl chloroformate at a low temperature, such as -15°C, in the presence of a base. The resultant dipeptide of formula (VII) is deprotected at the N-terminus, as described in Scheme II, by treatment with 4 M HCl in dioxane to afford the hydrochloride salt of formula (VIII). The coupling process described in Scheme I is repeated with subsequent nitrogen protected peptide residues followed by deprotection as described in Scheme II to the partially protected tetrapeptide of formula (IX). Peptide residue W is a differentially protected diamino residue such as, but not limited to, lysine, protected by a t-butyloxycarbonyl group at the α-amine and a benzyloxycarbonyl (Z) group at the ε-amine. The α-amine is then deprotected as described in Scheme II to provide the hydrochloride salt of the partially protected pentapeptide of formula (X). Compound (X) is acylated at the α-amine as shown in Scheme III wherein an acid chloride of formula (XI) is allowed to react with (X) at room temperature for 24 hours in an inert solvent in the presence of a base to provide Compound (XII). (I) is prepared as described by Scheme IV wherein Compound (XII) is treated with palladium hydroxide, cyclohexene, acetic acid in ethanol at reflux for 24 hours. The resulting residue can be chromatographed and the choice of eluting solvents will be readily apparent to those skilled in the art.

N^{α}(Y)W-ProTyrIleLeu•HOAc

(I)

wherein, Y and W are as defined above.

Processes in which the peptide derivatives of the invention can be obtained when X is an α-amino acid residue disubstituted at the α-carbon by alkyl groups of 1-6 carbons, optionally connected to form a carbocyclic or heterocyclic ring of 3-8 members in which the heteroatom is N, O, or S are illustrated in Scheme V. These compounds correspond to general formulas (II) and (III).

Y-X-Pro-Tyr-Ile-Leu

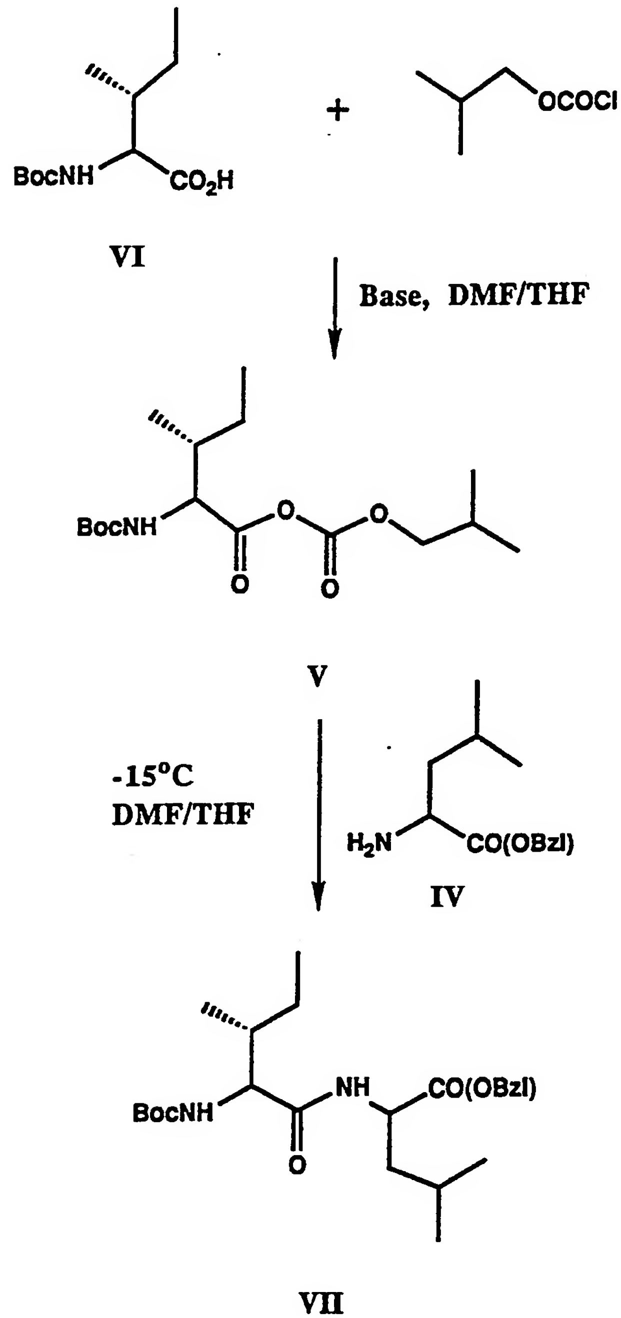
(II)

Y-W-X-Pro-Tyr-Ile-Leu

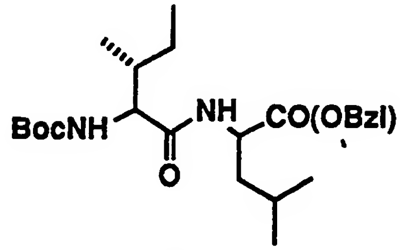
(III)

Illustrative examples of residue X are shown in Scheme VI.

Scheme I : Mixed Anhydride Coupling of Protected Amino Acids



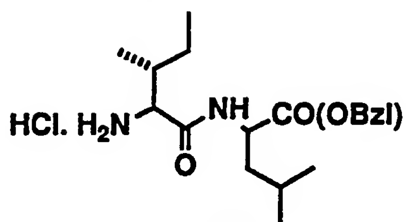
Scheme II: N-Terminal Deprotection of Intermediate Peptides



VII

4M HCl/Dioxane

25°C, 15 min



VIII

N^{α}(Boc)-N^{ε}(Z)-W-Pro-Tyr(OBzl)-Ile-Leu(OBzl)

IX

Scheme III: Attachment of the Bulky Lipophilic Group Y

N^{ε}(Z)-W-Pro-Tyr(OBzl)-Ile-Leu(OBzl). HCl

X

+

Y-Cl

XI

$\xrightarrow{\text{Base/}CH_2Cl_2\text{, 25°C, 24 hr}}$

N^{α}(Y)-N^{ε}(Z)-W-Pro-Tyr(OBzl)-Ile-Leu(OBzl)

XII

Scheme IV: Deprotection to Final Product

N^{α}(Y)-N^{ε}(Z)-W-Pro-Tyr(OBzl)-Ile-Leu(OBzl)

XII

$$\Big\downarrow \text{Cyclohexene, } Pd(OH)_2 \text{; EtOH/HOAc}$$

N^{α}(Y)-W-Pro-Tyr-Ile-Leu . HOAc

I

Scheme V: Preparation of Peptides Where X is a Disubstituted α-Amino Acid

Pro-Tyr(OBzl)-Ile-Leu(OBzl)

↓ *N*-Boc-X/ DCC/HOBT or Im_2CO

N-Boc-X-Pro-Tyr(OBzl)-Ile-Leu(OBzl)

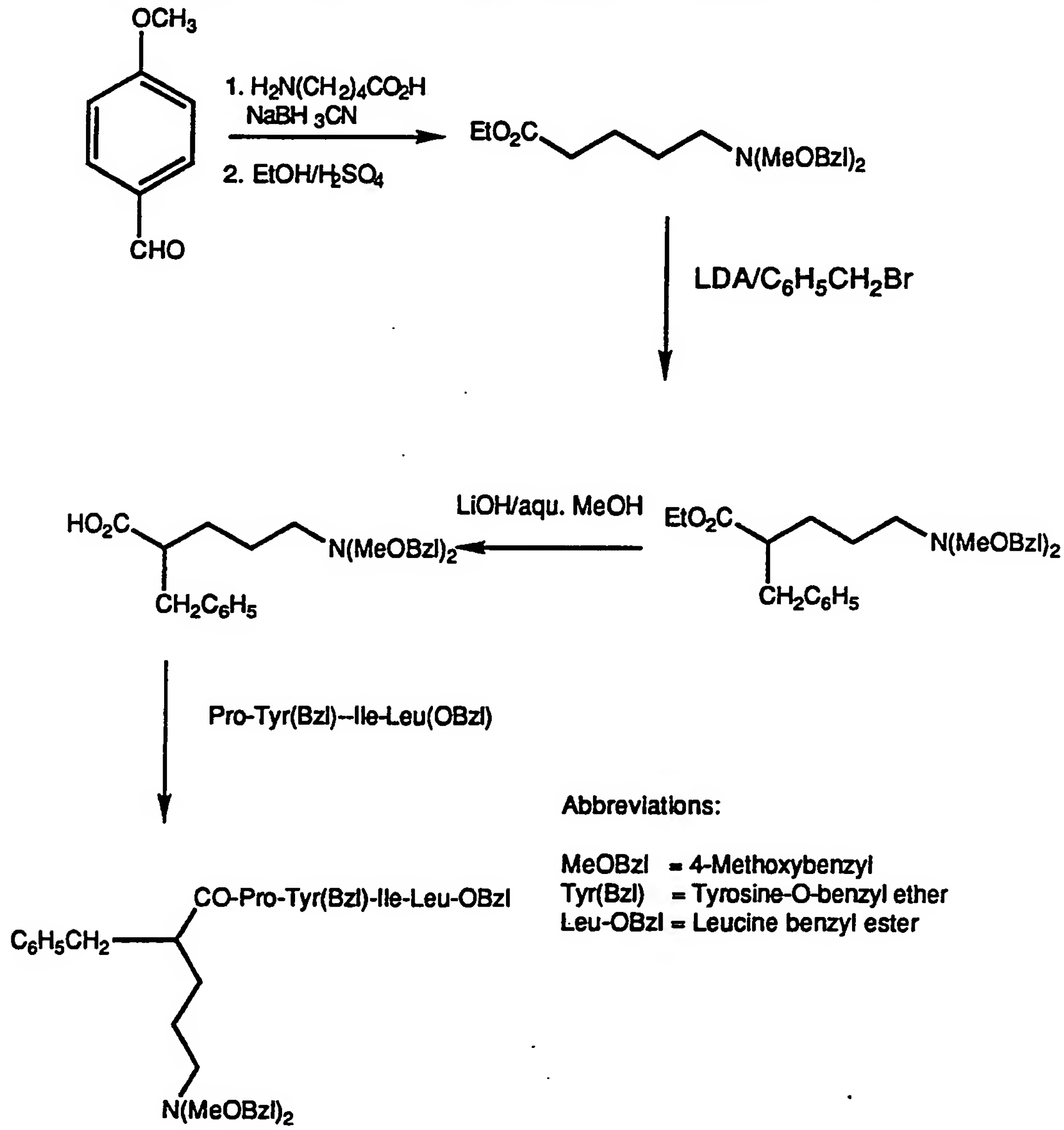
↙
1. HCl/Dioxan
2. YCl
3. H_2, Pd/C

Y-X-Pro-Tyr-Ile-Leu
II

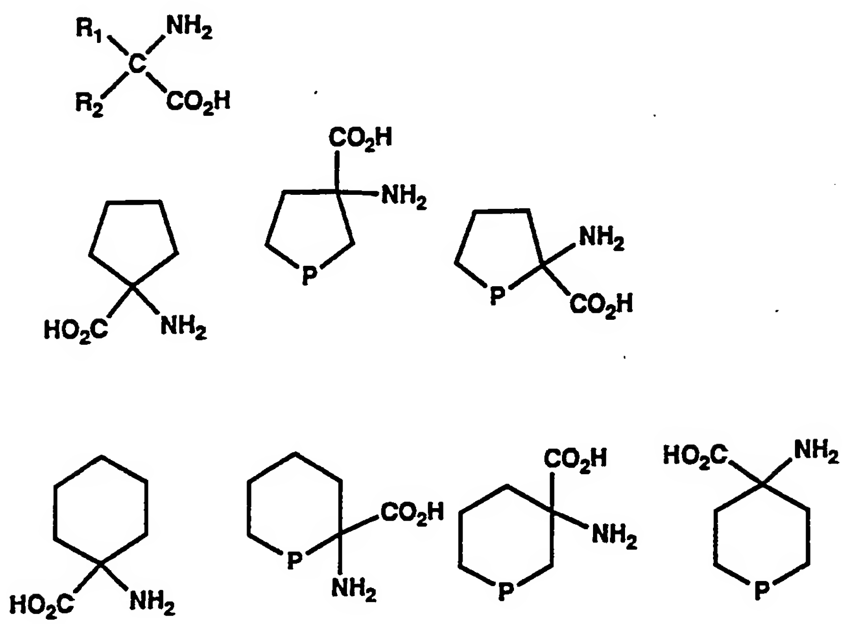
↘
1. HCl/Dioxan
2. N-Boc-W
3. HCl/Dioxan
4. YCl
5. H_2, Pd/C

Y-W-X-Pro-Tyr-Ile-Leu
III

Scheme VA: Preparation of Peptides Where the N-Terminal is an α-Substituted ω-Amino Acid



Scheme VI: Illustrative Examples of Disubstituted Amino Acid X



R_1, R_2 = alkyl of 1- 6 carbons
P = NH, O, S

Examples

In the following Examples, standard three-letter amino acid abbreviations are used to describe the peptides of the invention; "Z" represents the carbobenzyloxy protecting group; "Boc" represents the t-butoxycarbonyl protecting group; "OBzl" or "Bzl" represents O-benzyl, either the benzyl ether of tyrosine or the benzyl ester of a C-terminal amino acid; "$\psi[CH_2NH]$" and "$\psi[CH{=}CH]$" represent the replacement of the normal peptide amide bond (CONH) by methyleneimino (CH_2NH) or olefin (CH=CH) within a peptide chain. The starting amino acids used below were purchased from one of four commercially available sources: Sigma (St. Louis, MO), Bachem Bioscience, Inc. (Philadelphia, PA), Advanced Chemtech (Louisville, KY); and/or Peninsula Laboratories, Inc. (Belmont, CA).

Example 1

N^{α}-(1-Adamantanecarbonyl)-Arg-Pro-Tyr-Ile-Leu, Acetic Acid Salt (SEQ ID NO:1)

Synthesis of this peptide was based on standard solid-phase peptide synthesis methodology using a Beckman 990 MP peptide synthesizer with N-Boc leucine phenylacetamidomethyl (PAM) resin (1% divinyl benzene polystyrene) as the solid support. The Boc group was cleaved using 50% trifluoroacetic acid, 5% thioanisole in dichloromethane for 30 min at room temperature. Neutralization of the resulting ammonium salt was performed by treatment with diisopropylethylamine in dichloromethane. The amino acids were coupled in turn as their N-Boc derivatives using dicyclohexylcarbodiimide/1-hydroxybenzotriazole in dimethylformamide in a 2.5 fold excess. The side chain functionalities of Tyr

and Arg were protected by 2-bromobenzyloxycarbonyl and tosyl respectively.

The 1-adamantanecarbonyl group was coupled to the resin bound peptide by treatment with a five fold excess of 1-adamantanecarbonyl chloride and one equivalent of diisopropylethylamine in dimethylformamide. This treatment was repeated until all peptide amine groups were blocked with the adamantanecarbonyl groups.

The peptide was cleaved from the resin and the protecting groups were removed by treating the peptide resin with a solution of anisole in anhydrous HF (1:10). Purification of the deprotected peptide was accomplished by reverse phase high performance liquid chromatography.

Fast Atom Bombardment Mass Spectrometry (FAB-MS) (M+H) calc'd 823.51, found 823.45.

Example 2

N^{α}-(1-Adamantanecarbonyl)-Arg-Arg-Pro-Tyr-Ile-Leu, Acetic Acid Salt (SEQ ID NO:2)

This peptide was prepared according to the procedure described above in Example 1.

FAB-MS: (M+H) calc'd 979.61, found 979.53.

Example 3

N^{α}-(1-Adamantanecarbonyl)-Lys-Pro-Tyr-Ile-Leu, Acetic Acid Salt (SEQ ID NO:3)

Step A: N-Boc-Isoleucylleucine, Benzyl Ester (N-Boc-Ile-Leu(OBzl))

N-Boc-isoleucine (N-Boc-Ile•0.5 H_2O), 5.0 g (20.8 mmol) was dissolved in 110 ml of dry THF and chilled to -15°C in a dry ice acetone bath. A separate flask was charged with 8.19 g (20.8 mmol) of leucine benzyl ester tosylate (Tos•Leu(OBzl)), 40 ml DMF then chilled to

$-15°C$. 2.11 g (20.8 mmol) of N-methylmorpholine was added to the flask containing the N-Boc-Ile•0.5 H_2O followed by addition of 2.84 g (20.8 mmol) isobutyl chloroformate. This was allowed to stir for 5 min at $-15°C$. 2.11 g (20.8 mmol) of N-methylmorpholine was also added to the flask containing the Tos•Leu(OBzl) and DMF. This was transferred via cannula to the reaction vessel containing N-Boc-Ile•0.5 H_2O, N-methylmorpholine and THF. Reaction was allowed to stir at $-15°C$ for 0.5 hour then allowed to warm to room temperature and stir for 3 hours. Solvent was then stripped *in vacuo* and resulting residue was taken up in 300 ml ethyl acetate. This was extracted with 200 ml 5% aqueous bicarbonate, 200 ml H_2O, 200 ml 0.1 M HCl, and 200 ml H_2O. The organic layer was dried over magnesium sulfate, solvent stripped and resulting product chromatographed using 5% methanol in chloroform as solvent to give 8.7 g (96.3% yield) of N-Boc-Ile-Leu(OBzl), characterized as follows:

	MS(calc'd)	MS(found)
N-Boc-Ile-Leu(OBzl)	434	434

	Analysis Cal	Analysis Exp.
%C	66.33	66.15
%H	8.81	8.91
%N	6.45	6.80

^1H NMR (300 MHz, DMSO/TMS δ): 8.2(d, 1H, J=7.3HZ); 7.35(s, 5H); 6.7(d, 1H, J=9.2HZ); 5.1(s, 2H); 4.39(m, 1H); 3.8(t, 1H, J=8.4,8.4); 1.7-1.5(m, 4H); 1.39(s, 9H); 1.1(m, 1H); 0.9(d, 3H, J=2.9HZ); 0.8(m, 9H).

Step B: Isoleucylleucine, Benzyl Ester, Hydrochloride (Ile-Leu(OBzl)•HCl)

The compound synthesized in Step A above (8.7 g), was suspended in 44 ml (≈5 ml/g of substrate) 4 M HCl/Dioxane and stirred at room temperature for 15 min. Solvent then stripped to yield 7.30 g (98% yield) of Ile-Leu(OBzl).

	MS(calc'd)	MS(found)
Ile-Leu(OBzl)•HCl	334	334

^{1}H NMR (300 MHz, DMSO/TMS δ): 8.9(d, 1H, J=7.0HZ); 8.4(bs); 7.4(s, 5H); 5.1(s, 2H); 4.4(m, 1H); 3.7(d, 1H, J=5.5HZ); 1.9-1.4(m, 4H); 1.1(m, 1H); 0.9-0.8(m, 10H).

Step C: N-Boc-Tyr(OBzl)Ile-Leu(OBzl)

N-Boc-Tyr(OBzl) was coupled to the compound synthesized in Step B above using the procedure described in Step A above to yield 89.8% of N-Boc-Tyr(OBzl)-Ile-Leu(OBzl).

	MS(calc'd)	MS(found)
N-Boc-Tyr(OBzl)-Ile-Leu(OBzl)•1/2 H_2O	687	687

	Cal. w/1/2 mole hydrate	Exp.
%C	68.85	68.88
%H	7.70	7.75
%N	6.01	6.03

^{1}H NMR (300 MHz, DMSO/TMS δ): 8.4(d, 1H, J=7.3HZ); 7.7(d, 1H, J=8.8HZ); 7.4-7.3(m, 5H); 7.1(m, 2H); 6.9(m, 3H); 5.1(s, 2H); 5.05(s, 2H); 4.4-4.0(m, 3H); 2.9-2.6(m,

3H); 1.8-1.4(m, 3H); 1.3(s, 9H); 1.1(m, 1H); 0.8(m, 12H).

Step D: Tyr(OBzl)-Ile-Leu(OBzl)•HCl

9.5 g (13.8 mmol) of the compound synthesized in Step C above was deprotected according to the procedure described in Step B above to yield 8.5 g of Tyr(OBzl)-Ile-Leu(OBzl)•HCl.

	MS(calc'd)	MS(found)
Tyr(OBzl)-Ile-Leu(OBzl)•HCl	587	587

^{1}H NMR (300 MHz, DMSO/TMS δ): 8.75(d, 1H, J=8.8HZ); 8.6(d, 1H, J=7.3HZ); 8.2(bs, 2H); 7.4(m, 5H); 7.2(m, 2H); 6.9(m, 2H); 5.19(s, 2H); 5.05(s, 2H); 4.4(m, 1H); 4.2(m, 1H); 4.1(m, 1H); 3.4(m, 1H); 3.1(m, 1H); 2.9(m, 1H); 1.8-1.6(m, 4H); 1.1(m, 1H); 0.9-0.8(m, 7H).

Step E: N-Boc-Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:9)

1.90 g (8.8 mmol) of N-Boc-Pro was coupled to the compound synthesized in Step D above using the same procedure as described in Step A above to yield 3.3 g (48.0% yield) of N-Boc-Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:9).

	MS(calc'd)	MS(found)
SEQ ID NO:9	784	784

Analysis

	Cal	Exp.
%C	68.85	68.51
%H	7.70	7.92
%N	7.14	7.30

^1H NMR (300 MHz, DMSO/TMS δ): 8.4(bd, 1H); 8.0(bd, 1H); 7.79(d, 1H, J=8.1HZ); 7.4(m, 10H); 7.1(m, 2H); 6.8(d, 2H,J=8.7HZ); 5.1(s, 2H); 5.05(s, 2H); 4.6(m, 1H); 4.39(m, 1H); 4.2(t, 1H, J=8.5,8.4); 4.0(m, 1H); 3.2(m, 1H); 2.9(m, 1H); 2.7(m, 1H); 2.0(m, 1H); 1.8-1.4(m, 6H); 1.4(s, 3H); 1.1(s, 6H); 0.9-0.7(m, 9H).

Step F: Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:10)

3.3 g (4.2 mmol) of the product synthesized in Step E above was deprotected using same procedure as described in Step B above to yield 3.01 g (99.3% yield) of Pro-Tyr(OBzl)-Ile-Leu(OBzl)•HCl (SEQ ID NO:10).

	MS(calc'd)	MS(found)
SEQ ID NO:10	684	684

^1H NMR (300 MHz, DMSO/TMS δ): 9.7(bs, 1H); 8.79(d, 1H, J=8.7HZ); 8.4(d, 2H, J=7.3HZ); 8.1(d, 1H, J=8.8HZ); 7.4(m, 9H); 7.2(d, 2H, J=8.5HZ); 6.9(d, 2H, J=8.4HZ); 5.1(s, 2H); 5.05(s, 2H); 4.6(m, 1H); 4.4(m, 1H); 4.2(m, 1H); 4.1(m, 1H); 3.1(bs, 2H); 3.0(m, 1H); 2.8(m, 1H); 2.3(m, 1H); 1.9-1.4(m, 8H); 1.1(m, 1H); 0.9-0.7(m, 10H).

Step G: N^α-Boc,N^ε(Z)-Lys-Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:11)

1.74 g (4.6 mmol) of N^α-Boc,N^ε(Z)Lys was coupled to the product synthesized in Step F above using the same procedure as described above in Step A to yield 3.1 g (64.4% yield) of Compound N^α-Boc-N^ε(Z)-Lys-Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:11).

	MS(calc'd)	MS(found)
SEQ ID NO:11	1046	1046

	Analysis	
	Cal	Exp.
%C	67.66	67.39
%H	7.51	7.66
%N	8.02	7.87

^{1}H NMR (300 MHz, DMSO/TMS δ): 8.4(m, 1H); 7.8(m, 1H); 7.4-7.2(m, 14H); 7.1(d, 2H); 6.9(m, 2H); 5.15(s, 2H); 5.01(s, 2H); 5.0(s, 2H); 4.5-4.0(m, 5H); 3.6(m, 1H); 3.4(m, 1H); 3.0(m, 2H); 2.7(m, 1H); 2.0(m, 1H); 1.9-1.5(m, 9H); 1.4(s, 9H); 1.01(m, 1H); 0.9-0.7(m, 12H).

Step H: N^{ε}(Z)-Lys-Pro-Tyr(OBzl)-Ile-Leu(OBzl)•HCl (SEQ ID NO:12)

3.1 g (2.96 mmol) of the product synthesized as described in Part G above was deprotected according to the procedure described in Step B above to yield 2.9 g (99.6% yield) of SEQ ID NO:12.

	MS(calc'd)	MS(found)
SEQ ID NO:12	946	946

	Analysis	
	Cal	Exp.
%C	65.99	66.10
%H	7.13	7.30
%N	8.55	8.40

^{1}H NMR (300 MHz, DMSO/TMS δ): 8.4(m, 1H); 8.2(m, 2H); 8.05(m, 1H); 7.9(m, 1H); 7.4-7.2(m, 16H); 7.1(m, 3H); 6.9(m, 3H); 5.1(s, 2H); 5.05(s, 2H); 5.0(s, 2H); 4.5(m, 2H); 4.4(m, 1H); 4.25(m, 1H); 4.05(m, 1H); 3.0-2.7(m,

4H); 2.05(m, 1H); 1.8-1.3(m, 16H); 1.0(m, 1H); 0.9-0.7(m, 13H).

Step I: N^{α}-(1-Adamantanecarbonyl),N^{ε}(Z)-Lys-Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:13)

1.0 g (1.0 mmol) of the product synthesized in Step H above was dissolved in 50 ml CH_2Cl_2 and 0.3 ml (2.1 mmol) of triethylamine. This was chilled to 0°C and 202.3 mg (1.0 mmol) of 1-adamantanecarbonyl chloride dissolved in 5 ml CH_2Cl_2 was added. Reaction mixture was allowed to come to room temperature and stirred for 24 hours and then poured into H_2O and layers separated. Organic layer washed 3 x 50 ml H_2O and 2 x 50 ml saturated NaCl then dried over magnesium sulfate. Solvent stripped and residue chromatographed using 5% methanol in chloroform as solvent. 1.05 g of N^{α}-(1-adamantanecarbonyl)-N^{ε}-(Z)-Lys-Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:13) was isolated.

N^{α}-(1-Adamantanecarbonyl),N^{ε}(Z)-Lys-Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:13)

	MS(calc'd)	MS(found)
SEQ ID NO:13	1108	1108

^1H NMR (300 MHz, DMSO/TMS δ): 7.4-7.2(m, 10H); 7.1(m, 2H); 6.9(m, 2H); 6.7-6.4(m, 2H); 5.1(m, 6H); 3.5(m, 1H); 3.1(m, 1H); 3.2(m, 4H); 2.1(m, 6H); 1.9(bs, 6H); 1.8(s, 4H); 1.8-1.2(m, 22H); 1.1(m, 2H); 0.9(m, 11H).

Step J: N^{α}-(1-Adamantanecarbonyl)-Lys-Pro-Tyr-Ile-Leu (SEQ ID NO:3)

1.05 g (0.95 mmol) of the product synthesized in Step I above was mixed with 50 ml ethanol, 10 ml of cyclohexene, 0.054 ml (0.95 mmol) of glacial acetic acid

and 100 mg 20% palladium hydroxide on carbon. Reaction was refluxed for 24 hours. After which the reaction mixture was cooled and catalyst removed by filtration through a pad of Celite®. Solvent stripped *in vacuo* to give 0.68 g (86.2% yield) of N^{α}-(1-adamantanecarbonyl)-Lys-Pro-Tyr-Ile-Leu (SEQ ID NO:3). mp 120-122°C.

	MS(calc'd)	MS(found)
SEQ ID NO:3	794	794

Analysis

	Cal	Exp.
%C	63.22	63.36
%H	8.20	8.50
%N	9.84	9.89

^{1}H NMR (300 MHz, DMSO/TMS δ): 8.0(m, 1H); 7.4(m, 1H); 7.3(m, 1H); 7.0(d, 2H); 6.6(m, 2H); 4.6-4.2(m, 3H); 4.0(m, 2H); 3.0(m, 1H); 2.8-2.6(m, 2H); 2.0(m, 3H); 1.9(s, 1H); 1.8(m, 9H); 1.6(s, 9H); 1.4-1.2(m, 3H); 1.1(t, 2H); 0.9(m, 11H).

Example 4

N^{α}-(2-Norbornaneacetyl)-Lys-Pro-Tyr-Ile-Leu, Acetic Acid Salt (SEQ ID NO:15)

Step A: N^{α}-(2-Norbornaneacetyl),N^{ε}(Z)-Lys-Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:14)

0.15 g (0.15 mmol) of the product from Example 3, Step H above was coupled to 2-norbornaneacetyl chloride according to the procedure des::ribed above for the preparation of the compound of :xample 3, Step I. 0.15 g (92% yield) of N^{α}-(2-norbornaneacetyl)-N^{ε}-(Z)-Lys-Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:14) was isolated.

	MS(calc'd)	MS(found)
SEQ ID NO:14	1082	1082

Analysis

	Cal	Exp.
%C	69.87	69.98
%H	7.58	7.73
%N	7.76	7.85.

Step B: N^{α}-(2-Norbornaneacetyl)-Lys-Pro-Tyr-Ile-Leu, Acetic Acid Salt (SEQ ID NO:15)

0.14 g (0.13 mmol) of the compound synthesized above in Example 4, Step B was deprotected according to the procedure described above in Example 3, Step J to give 0.11 g (100% yield) of N^{α}(2-norbornaneacetyl)-Lys-Pro-Tyr-Ile-Leu (SEQ ID NO:15) acetic acid salt.

N^{α}-(2-Norbornaneacetyl)-Lys-Pro-Tyr-Ile-Leu(SEQ ID NO:15)$\cdot CH_3CO_2H$

MS(calc'd)	MS(found)	mp 87-90°C
768	768.	

Example 5

$N^{\alpha}(CF_3(CF_2)_6CO)$-Lys-Pro-Tyr-Ile-Leu, Acetic Acid Salt (SEQ ID NO:17)

Step A: $N^{\alpha}(CF_3(CF_2)_6CO)$,$N^{\varepsilon}$(Z)-Lys-Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:16)

0.1 g (0.1 mmol) of the product synthesized above in Example 3, Step H was coupled to perfluorooctanoyl chloride according to the procedure as described above for the preparation of the compound synthesized in Example 4, Step A above. 0.13 g (96.8% yield) of N^{α}-(perfluorooctanoyl)-N^{ε}(Z)-Lys-Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:16) was isolated.

$N^{\alpha}(CF_3(CF_2)_6CO),N^{\varepsilon}(Z)$-Lys-Pro-Tyr(Bzl)-Ile-Leu(OBzl) (SEQ ID NO:16)

MS(calc'd)	MS(found)	mp 120-125°C
1342	1342.	

Step B: $N^{\alpha}(CF_3(CF_2)_6CO)$-Lys-Pro-Tyr-Ile-Leu, Acetic Acid Salt (SEQ ID NO:17)

0.13 g of the product synthesized in Example 5, Step A above was deprotected according to the procedure described above Example 3, Step J to produce 90 mg (85.4% yield) of N^{α}-(perfluorooctanoyl)-Lys-Pro-Tyr-Ile-Leu (SEQ ID NO:17), acetic acid salt.

SEQ ID NO:17•CH_3CO_2H

MS(calc'd)	MS(found)	mp 150-152°C
1028	1028.	

Example 6

N^{α}-(cis-Bicyclo(3.3.0)Octane-2-Carbonyl)-Lys-Pro-Tyr-Ile-Leu, Acetic Acid Salt (SEQ ID NO:5)

Step A: N^{α}(cis-Bicyclo(3.3.0)Octane-2-carbonyl), N^{ε}(Z)-Lys-Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:18)

0.15 g (0.15 mmol) of the product synthesized above in Example 3, Step H was coupled to cis-bicyclo(3.3.0)octane-2-carbonyl chloride according to the procedure described above for the preparation of the compound synthesized in Example 4, Step A above. 0.15 g (9[illegible]4% yield) N^{α}-(cis-bicyclo(3.3.0)octane-2-carbonyl)-N^{ε}(Z)-Lys-Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:18), a clear oil, was isolated.

N^{α}-(cis-Bicyclo(3.3.0)octane-2-carbonyl]),N^{ϵ}(Z)-Lys-Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:18)

MS(calc'd)	MS(found)
1082	1082.

Step B: N^{α}-(cis-Bicyclo(3.3.0)-Octane-2-Carbonyl)-Lys-Pro-Tyr-Ile-Leu, Acetic Acid Salt (SEQ ID NO:5)

0.15 g (0.14 mmol) of the product synthesized in Example 6, Step A was deprotected according to the procedure described above in Example 3, Step J to yield 0.12 g of N^{α}-(cis-bicyclo(3.3.0)-octane-2-carbonyl)-Lys-Pro-Tyr-Ile-Leu (SEQ ID NO:5), acetic acid salt.

HRMS (M+H)	Measured	Calculated
	769.49	769.49

m.p. 170-173°C.

Example 7

N^{α}-(1-Adamantanecarbonyl)-Lys-Pro-ψ[CH_2NH]-Tyr-Ile-Leu, Acetic Acid Salt (SEQ ID NO:4)

Step A: N-Boc-Prolinal

7.58 g (59.7 mmol) of oxalyl chloride was mixed with 100 ml CH_2Cl_2 and chilled to -78°C. 8.93 g (114.4 mmol) of dry methylsulfoxide was added to the mixture and allowed to stir for 15 min. 10.0 g (50 mmol) of L-Prolinol (dissolved in 25 ml CH_2Cl_2) was added and allowed to stir for another 15 min. 11.66 g (114.4 mmol) of triethylamine was added and reaction allowed to come to room temperature and stir for 24 hours. Reaction mixture was then poured into H_2O and layers separated. Organic layer washed 3 x 100 ml H_2O, 2 x 100 ml saturated NaCl and dried over magnesium sulfate.

Solvent stripped in vacuo to give 9.0 g (90.5% yield of N-Boc-prolinal, a clear oil.

	MS(calc'd)	MS(found)
N-Boc-Prolinal	199	199

^{1}H NMR (300 MHz, $CDCl_3$/TMS δ): 9.5(s, 1H); 4.2(m, 1H); 3.5(m, 2H); 2.2-1.8(m, 4H); 1.1(s, 3H); 1.45(s, 3H); 1.4(s, 3H).

Step B: N-Boc-Pro-ψ[CH_2NH]Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:19)

0.7 g (3.5 mmol) of N-Boc-prolinal, 2.0 g (3.2 mmol) of the product of Example 3, Step D, and 0.287 g (3.5 mmol) of sodium acetate were added to 50 ml of a 2% acetic acid/DMF solution. 2.0 g (32 mmol) of $NaBH_3CN$ was then added slowly and reaction allowed to stir at room temperature for 24 hours. Reaction mixture poured into aqueous K_2CO_3 (pH 10) and extracted 3 x 100 ml with ethyl acetate. Organic layer dried over magnesium sulfate, solvent stripped in vacuo and residue chromatographed using 1:1 ethyl acetate:hexane as solvent. 2.0 g (81.2% yield) of the following protected pseudo-tetrapeptide was isolated.

	MS(calc'd)	MS(found)
SEQ ID NO:19	770	770

Step C: Pro-ψ[CH_2NH]Tyr(OBzl)-Ile-Leu(OBzl), Hydrochloride Salt (SEQ ID NO:20)

2.0 g of the product synthesized above in Example 7, Step B was deprotected according to the procedure described above in Example 3, Step B to provide 1.46 g of the product described in Example 7, Step C. This was then coupled to N^{α}Boc,N^{ε}(Z)-Lys according to the

procedure described above in Example 3, Step G to give the pseudo-tetrapeptide as described below in Step D. This in turn was deprotected according to the procedure described above in Example 3, Step B to give the product described below in Example 7, Step E, which was then coupled to 1-adamantanecarbonyl chloride as described above in Example 3, Step I to give the product described in Example 7, Step F. Final deprotection of the product described below in Example 7, Step G was performed in the same way as described above in Example 3, Step J to give the product described below in Example 7, Step G.

Step C: Pro-ψ[CH_2NH]Tyr(OBzl)-Ile-Leu(OBzl)•HCl (SEQ ID NO:20)

MS(calc'd)	MS(found)
670	670

^{1}H NMR (300 MHz, DMSO/TMS δ): 10.0(m, 1H); 9.4(bs); 8.8(bs, 1H); 8.55(m, 1H); 7.4(m, 10H); 7.2(d, 2H); 6.9(d, 2H); 5.15(s, 2H); 5.05(s, 2H); 4.4-4.1(m, 5H); 3.9(m, 1H); 3.7(m, 1H); 3.2(m, 4H); 3.0(m, 1H); 2.1(m, 1H); 1.9(m, 2H); 1.8-1.5(m, 6H); 1.4(m, 1H); 1.1(m, 1H); 1.0-0.7(m, 12H).

Step D: N^{α}-Boc,N^{ε}(Z)-Lys-Pro-ψ[CH_2NH]Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:21)

MS(calc'd)	MS(found)
1032	1032

^{1}H NMR (300 MHz, DMSO/TMS δ): 8.4(d, 1H); 7.9(m, 1H); 7.4(m, 12H); 7.2(m, 1H); 7.1(d, 2H); 6.8(m, 3H); 5.1(s, 2H); 5.05(s, 2H); 5.0(s, 2H); 4.5(m, 1H); 4.2(m, 1H); 3.9(m, 1H); 3.7(m, 1H); 3.0(m, 2H); 2.8(m, 1H); 2.6(m,

1H); 2.4(m, 1H); 2.2(m, 1H); 1.8-1.5(m, 10H); 1.4(s, 10H); 1.0(m, 1H); 0.9-0.7(m, 12H).

Step E: N^{ε}(Z)-Lys-Pro-ψ[CH_2NH]Tyr(OBzl)-Ile-Leu(OBzl)•HCl (SEQ ID NO:22)

MS(calc'd)	MS(found)
932	932

^{1}H NMR (300 MHz, DMSO/TMS δ): 9.6(bs, 1H); 9.0(bs, 1H); 8.7(m, 1H); 8.5(m, 1H); 8.2(m, 3H); 7.4(m, 15H); 7.1(d, 2H); 6.9(d, 2H); 5.1(s, 2H); 5.05(s, 2H); 5.0(s, 2H); 4.4-4.0(m, 4H); 3.7(s, 1H); 3.6(m, 1H); 3.1(m, 1H); 3.0(m, 1H); 2.7(m, 1H); 2.0-1.2(m); 0.9(m, 12H).

Step F: N^{α}(1-Adamantanecarbonyl),N^{ε}(Z)-Lys-Pro-ψ[CH_2NH]Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:23)

MS(calc'd)	MS(found)
1094	1094

TLC R_f. = 0.3 (1:5 MeOH:$CHCl_3$)

Step G: N^{α}(1-Adamantanecarbonyl)-Lys-Pro-ψ[CH_2NH]Tyr-Ile-Leu•CH_3CO_2H (SEQ ID NO:4)

MS(calc'd)	MS(found)
780	780

	Analysis	
	Cal	Exp.
%C	61.43	61.05
%H	8.57	8.30
%N	10.00	9.50.

Example 8

N^{α}-(1-Adamantanecarbonyl)-Orn-Pro-Tyr-Ile-Leu, Acetic Acid Salt (SEQ ID NO:6)

Step A: The partially protected tetrapeptide Pro-Tyr(Bzl)-Ile-Leu(OBzl)•HCl made in Example 3, Step F (SEQ ID NO:10) was coupled to N^{δ}-(Z),N^{α}-Boc-Ornithine using standard isobutyl chloroformate/N-methyl morpholine chemistry to yield N^{δ}-(Z),N^{α}-Boc-Orn-Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:24) (62%) as a colorless solid after silica gel chromatography (EtOAc/hexanes).

MS-DCI (NH_3): 1033 (M+H, 18%), 933 (M+H-Boc, base)

$[\alpha]_D^{25}$ -57.6° (c = 0.604, CHCl3)

Anal. Calc'd. for $C_{58}H_{76}N_6O_{11}$:

	C 67.42	H 7.41	H 8.13
Found:	67.20	7.47	7.97.

Step B: The fully protected pentapeptide synthesized in Step A above was treated with 4 M HCl in dioxane under standard conditions, then precipitated by dilution with diethyl ether to yield N^{δ}-(Z)-Orn-Pro-Tyr(OBzl)-Ile-Leu(OBzl)•HCl (SEQ ID NO:25) (93%) as a colorless solid.

MS-DCI (NH_3): 933 (M+H, base)

$[\alpha]_D^{25}$ -37.0° (c = 0.606, MeOH).

Step C: The amine salt synthesized above in Step B (0.30 g, 0.31 mmol), triethylamine (95 µL, 0.68 mmol),

1-adamantanecarbonyl chloride (65 mg, 0.31 mmol), and dichloromethane were stirred at ambient temperature for 1.75 hour. The mixture was diluted with ethyl acetate (30 mL), and extracted with 1 M HCl (2 mL), water (3 mL), sat. aq. sodium carbonate (3 mL), and brine (3 mL), then dried ($MgSO_4$), filtered, and concentrated _in vacuo_. After drying further under high vacuum, N^{δ}-(Z),N^{α}-(1-adamantanecarbonyl)-Orn-Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:26), 0.34 g (quantitative) was obtained as a colorless solid.
MS-DCI (NH_3): 1095 (M+H, 15%), 161 (base)
R_f (5% methanol/chloroform) = 0.48

Step D: The protected N^{α}-1-adamantanecarbonyl pentapeptide synthesized above in Step B (0.33 g, 0.30 mmol) was deprotected using the standard catalytic transfer hydrogenation conditions (20% palladium hydroxide on carbon, cyclohexene, acetic acid, ethanol at reflux) to provide N^{α}-(1-adamantanecarbonyl)-Orn-Pro-Tyr-Ile-Leu•HOAc (SEQ ID NO:6) (0.24 g, 95%) as a colorless solid.
MS-DCI (NH_3): 781 (M+H, base), 505 (45%), 408 (64%), 356 (82%), 277 (70%)
$[\alpha]_D^{25}$ -52.3° (c = 0.614, MeOH).

Example 9

N^{α}-(1-Adamantanecarbonyl)-Lys-Pro-Trp-Ile-Leu, Acetic Acid Salt (SEQ ID NO:7)

Step A: Ile-Leu(OBzl)•HCl synthesized above in Example 3, Step B was coupled to N-Boc-Trp under standard isobutyl chloroformate/N-methyl morpholine conditions to yield N-Boc-Trp-Ile-Leu(OBzl) (97%) as a colorless solid after silica gel chromatography (ethyl acetate/hexanes).
MS-DCI (NH_3): 638 ($M+NH_4$, base), 621 (M+H, 42%)
$[\alpha]_D^{25}$ -32.4° (c = 0.602, $CHCl_3$).

Anal. Calc'd. for $C_{35}H_{48}N_4O_6$:

	C	H	N
	67.72	7.79	9.03
Found:	67.81	7.98	8.76.

Step B: The N-Boc group of the compound described above in Step A (2.8 g, 4.5 mmol) was cleaved by stirring with 4 M HCl in dioxane/anisole/1,2-ethanedithiol (98:1:1, 10 mL) at ambient temperature under a Drierite tube for 1 hour. The product was precipitated by dilution with diethyl ether, collected by filtration, and dried at 56°C under high vacuum for 2 hours to yield Trp-Ile-Leu(OBzl)•HCl (2.24 g, 89%) as a white solid.

MS-DCI (NH_3): 521 (M+H, base)

$[\alpha]_D^{25}$ -24.5° (c = 0.600, MeOH)

Anal. Calc'd. for $C_{30}H_{40}N_4O_4$•HCl•1/2 H_2O:

	C	H	N	Cl
	63.65	7.48	9.90	6.26
Found:	63.29	7.49	9.61	6.36.

Step C: The partially protected tripeptide synthesized above in Step B was coupled to N-Boc-Pro under standard conditions to yield N-Boc-Pro-Trp-Ile-Leu(OBzl) (SEQ ID NO:27) (96%) as a colorless solid after silica gel chromatography (ethyl acetate).

MS-DCI (NH_3): 735 (M+NH_4, 90%), 718 (M+H, base)

$[\alpha]_D^{25}$ -89.7° (c = 0.614, chloroform)

Anal. Calc'd. for $C_{40}H_{55}N_5O_7$•1/2 H_2O:

	C	H	N
	66.09	7.77	9.63
Found:	66.34	7.76	9.49.

Step D: The N-Boc group of tetrapeptide prepared in Step C above was cleaved as in the previous example to

yield Pro-Trp-Ile-Leu(OBzl)•HCl (SEQ ID NO:28) (88%) as a solid which contained ~10% of an unknown impurity.
MS-DCI (NH_3): 721 (M+H, est. 10%, unknown by-product), 617 (M+H, base, est. 90%, desired product).

Step E: The crude tetrapeptide amine salt prepared above in Step D was coupled to N^{ε}-Cbz,N^{α}-Boc-Lys under standard conditions to yield N^{ε}-(Z),N^{α}-Boc-Lys-Pro-Trp-Ile-Leu(OBzl) (SEQ ID NO:29) (66%) as a colorless solid after silica gel chromatography (ethyl acetate/hexanes).
MS-DCI (NH_3): 997 (M+NH_4, 66%), 980 (M+H, base), 880 (M+H-Boc, 46%).
$[\alpha]_D^{25}$ -76.8° (c = 0.608, chloroform)
Anal. Calc'd. for $C_{54}H_{73}N_7O_{10}$:

	C	H	N
	66.17	7.51	10.00
Found:	66.20	7.51	9.87.

Step F: The N-Boc group of pentapeptide prepared above in Step E was cleaved as described above to yield N^{ε}-Cbz-Lys-Pro-Trp-Ile-Leu(OBzl)•HCl (SEQ ID NO:30) (87%) as a light brown solid.
MS-DCI (NH_3): 880 (M+H, base)
R_f (10% methanol/chloroform) = 0.16.

Step G: The pentapeptide amine salt prepared above in Step F was acylated by 1-adamantanecarbonyl chloride as described above to yield N^{ε}-Cbz,N^{α}-(1-adamantane-carbonyl)-Lys-Pro-Trp-Ile-Leu(OBzl) (SEQ ID NO:31) (quant.) as a tan solid.
MS-DCI (NH_3): 1059 (M+NH_4, 15%), 1042 (M+H, base)
R_f (5% methanol/chloroform) = 0.20.

Step H: The compound prepared in Step G above was deprotected using standard catalytic transfer hydrogenation conditions to yield N^{α}-(1-

adamantanecarbonyl)-Lys-Pro-Trp-Ile-Leu•HOAc (SEQ ID NO:7) as a pale tan solid.

MS-DCI (NH_3): 818 (M+H, Base)

R_f (chloroform/methanol/benzene/water 8:8:8:1) = 0.44.

Example 10

N^{α}-(1-Adamantanecarbonyl)-Lys-Pro-Tyr-(S)-2-phenylglycyl-Leu, Acetic Acid Salt (SEQ ID NO:8)

Step A: (S)-N-Boc-2-phenylglycine was coupled with Leu(OBzl) p-toluenesulfonic acid salt under standard conditions to yield (S)-N-Boc-2-phenylglycyl-Leu(OBzl) (66%) as a pale yellow solid after silica gel chromatography (ethyl acetate/hexanes).

m.p. 116-118°C

MS-DCI (NH_3): 455 (M+H, base)

$[\alpha]_D^{25}$ +52.3° (c = 0.620 $CHCl_3$)

Anal. Calc'd. for $C_{26}H_{34}N_2O_5$:

	C 68.70	H 7.54	N 6.16
Found:	68.75	7.48	6.03.

Step B: The N-Boc group of the compound prepared above in Step A was cleaved under standard 4 <u>M</u> HCl in dioxane conditions to yield (S)-2-phenylglycyl-Leu(OBzl)•HCl (quant.).

m.p. 200-201°C

MS-DCI (NH_3): 355 (M+H, base)

$[\alpha]_D^{25}$ +29.8° (c = 0.534, methanol)

Step C: Dipeptide salt of the compound prepared above in Step B was coupled to N-Boc-Tyrosine Benzyl Ether (N-Boc-Tyr(OBzl)) under standard conditions to yield N-Boc-Tyr(OBzl)-(S)-2-phenylglycyl-Leu(OBzl) (89%) as a glassy solid.

MS-DCI (NH_3): 725 (M+NH_4, base), 708 (M+H, 14%)

$[\alpha]_D^{25}$ +14.3° (c = 0.608, chloroform)

Anal. Calc'd. for $C_{42}H_{49}N_3O_7$:

	C	H	N
	C 71.26	H 6.98	N 5.94
Found:	71.07	7.00	5.64.

Step D: The N-Boc group of tripeptide prepared above in Step C was cleaved under standard conditions to yield Tyr(OBzl)-(S)-2-phenylglycyl-Leu(OBzl)•HCl (93%) as a colorless solid.

MS-DCI (NH_3): 608 (M+H, base)

$[\alpha]_D^{25}$ +34.8° (c = 0.630, methanol)

Anal. Calc'd. for $C_{37}H_{41}N_3O_5$•HCl:

	C	H	N	Cl
	C 68.98	H 6.57	N 6.52	Cl 5.50
Found:	68.70	6.54	6.39	5.74.

Step E: Tripeptide amine salt prepared above in Step D was coupled to N-Boc-Pro under standard conditions to yield N-Boc-Pro-Tyr(OBzl)-(S)-2-phenylglycyl-Leu(OBzl) (SEQ ID NO:32) (99%) as a colorless solid after silica gel chromatography (ethyl acetate/hexanes).

MS-DCI (NH_3): 822 (M+NH_4, base), 805 (M+H, 50%)

$[\alpha]_D^{25}$ -15.2° (c = 0.558, chloroform)

Anal. Calc'd. for $C_{47}H_{56}N_4O_8$:

	C	H	H
	C 70.13	H 7.01	H 6.96
Found:	70.10	6.93	6.74.

Step F: The N-Boc group of tetrapeptide prepared above in Step E was cleaved under standard conditions to yield Pro-Tyr(OBzl)-(S)-2-phenylglycyl-Leu(OBzl)•HCl (SEQ ID NO:33) (95%) as a colorless solid.

MS-DCI (NH_3): 705 (M+H, base), 615 (M+H, 10%)

$[\alpha]_D^{25}$ +9.27° (c = 0.604, methanol)

Anal. Calc'd. for $C_{42}H_{48}N_4O_6$•HCl•1/2 H_2O:

	C	H	H	Cl
	C 67.23	H 6.72	H 7.47	Cl 4.72
Found:	67.56	6.53	7.41	5.01.

Step G: The tetrapeptide amine salt prepared above in Step F was coupled with N^{ϵ}-Cbz-N^{α}-Boc-Lys under standard conditions to yield N^{ϵ}-(Z),N^{α}-Boc-Lys-Pro-Tyr(OBzl)-(S)-2-phenylglycyl-Leu(OBzl) (SEQ ID NO:34) (76%) as a colorless solid after silica gel chromatography (ethyl acetate/hexanes).

MS-DCI (NH_3): 1084 ($M+NH_4$, 52%), 1067 (M+H, 35%), 967 (M+H-Boc, base)

$[\alpha]_D^{25}$ -16.6° (c = 0.596, chloroform)

Anal. Calc'd. for $C_{61}H_{74}N_6O_{11}$:

	C 68.65	H 6.99	H 7.87
Found:	68.53	7.02	7.74.

Step H: The N^{α}-Boc group of pentapeptide prepared above in Step G was cleaved under standard conditions to yield N^{ϵ}-Cbz-Lys-Pro-Tyr(OBzl)-(S)-2-phenylglycyl-Leu(OBzl)•HCl (SEQ ID NO:35) (91%) as a colorless solid.

MS-DCI (NH_3): 967 (M+H, 90%), 608 (base)

$[\alpha]_D^{25}$ -13.6° (c = 0.604, methanol).

Step I: The pentapeptide amine salt prepared above in Step H was acylated with 1-adamantanecarbonyl chloride under usual conditions to yield N^{ϵ}-(Z),N^{α}-(1-adamantanecarbonyl)-Lys-Pro-Tyr(OBzl)-(S)-2-phenylglycyl-Leu(OBzl) (SEQ ID NO:36) (quant.) as a colorless solid.

MS-DCI (NH_3): 1146 ($M+NH_4$, 32%), 1129 (M+H, base)

R_f (5% methanol/chloroform) = 0.30.

Step J: Deprotection of the product prepared above in Step I under standard catalytic transfer hydrogenation conditions provided N^{α}-(1-adamantanecarbonyl)-Lys-Pro-Tyr-(S)-2-phenylglycyl-Leu•HOAc (SEQ ID NO:8) (99%) as a colorless solid.

MS-DCI (NH_3): 815 (M+H, base), 568 (18%)

$[\alpha]_D^{25}$ -38.9° (c = 0.606, methanol)
R_f (chloroform/methanol/benzene/water 8:8:8:1) = 0.19.

Example 11

4-(1'-Adamantanecarbamido)-4-Piperidinecarbonyl-Pro-Tyr-Ile-Leu, Acetic Acid Salt (SEQ ID NO:40)

Step A: 1-Benzyl-4-cyano-4-aminopiperidine

1-Benzyl-4-piperidone, 10.5 g (56.7 mmoles), was treated with 8 ml NH_4OH, 3.34 g (62.36 mmoles), 3.74 g (58 mmoles) KCN in 10 ml water at 60°C for 16 hours and then at 80°C for 4.5 hours. After cooling the product was extracted with ethyl ether (100 ml twice), the combined ether extracts were washed with water and brine, dried over sodium sulfate and concentrated _in vacuo_. The resulting solid was extracted with hot hexanes (3 x 200 ml). Cooling of the hexanes extracts at 0°C afforded 10 g, 84% yield, of the product as white crystals mp 76.8-77.2°C.

NMR: 7.3(s, 5H); 3.55(s, 2H); 2.82(m, 2H); 2.35(m, 2H); 2.0(m, 2H); 1.8(m, 4H).

MS: 217 (M+2), 216 (M+1), 215 (M), 189 (100%), (M-28).

Anal. Calcd.:	C 72.52	H 7.96	N 19.52
Found:	72.63	7.93	19.62.

Step B: 1-Benzyl-4-(1'-Adamantane)Carbamidopiperidine-4-Carboxylic Acid

0.5 g (2.32 mmoles) of the product of Step A was treated with 0.475 g (2.32 mmoles) 1-adamantanecarbonyl chloride in the presence of 2.32 (mmoles) N-methylmorpholine in CH_2Cl_2 at 25°C for 16 hours and then at reflux for 2 hours. Then the reaction mixture was poured into a separatory funnel containing 200 ml EtOAc and 100 ml 5% $NaHCO_3$. The organic layer was separated and washed with brine, dried over Na_2SO_4 and concentrated _in vacuo_ to give 1-benzyl-4-(1'-

adamantane)carbamido-4-cyano-piperidine as a white solid. 0.51 g (1.35 mmoles) of the split was dissolved in 3 ml concentrated HCl and 3 ml water and heated at 70°C for 2 hours. The solid precipitate was filtered off under vacuum to give 0.5 g of a 4:1 mixture of product and starting material, by NMR analysis, after drying under vacuum, as the hydrochloride salt.

NMR (CD_3OD)δ: 7.44-7.60(m, 5H); 4.40(s, 2H); 3.4-3.5(m, 2H); 3.05-3.15(t, 2H); 2.50-2.60(d, 2H); 2.20-2.35(m, 2H).

Step C: 1-Benzyl-4-(1'-Adamantane)Carbamidopiperidine-4-Carboxy-Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:39)

Five hundred milligrams (0.69 mmoles) HCl•Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:10) and 0.5 of the crude product obtained in Step B above were treated with 142.5 mg (0.69 mmoles) DCC, 108 mg (0.69 mmoles) 1-hydroxybenzotriazole, and 0.09 ml (0.69 mmoles) N-methylmorpholine in 8 ml DMF at 0°C for 1 hour and then at 25°C for 72 hours. The mixture was poured into a separatory funnel containing 200 ml EtOAc and 60 ml 5% $NaHCO_3$. The organic layer was washed with water and brine, dried over Na_2SO_4 and concentrated <u>in vacuo</u> to a solid. This was chromatographed on silica gel using 0.5 % NH_4OH/5%/CH_3OH/CH_2Cl_2 to give 610 mg of product, 83% yield, mp 81.9-83.5°C.

NMR ($CDCl_3$)δ: 7.60-7.70(d, 1H); 7.20-7.40(m, 16H); 7.15-7.20(d, 2H); 7.10-7.14(d, 1H); 6.80-6.85(d, 2H); 6.02(s, 1H); 5.15(s, 2H); 5.00(s, 2H); 4.50-4.65(m, 2H); 4.24-4.28(t, 1H); 4.05-4.10(br s, 1H); 3.20-3.65(m, 5H); 3.20-3.25(m, 1H); 3.00-3.05(d, 1H); 2.70-2.85(m, 2H); 2.40-2.55(m, 1H); 2.25-1.00(m, 29H); 0.80-1.00(m, 12H).

MS(NH_3): 1064 (M+2, 65%); 1063 (M+1, 100%).

Analysis for $C_{64}H_{82}N_6O_8$•1/2(H_2O):

Calc'd:	C 71.68	H 7.80	N 7.84
Found:	71.67	7.69	7.70.

Step D: 4-(1'-Adamantane)Carbamidopiperidine-4-Carbonyl-Pro-Tyr-Ile-Leu•AcOH (SEQ ID NO:40)

200 mg (0.19 mmoles) of the product obtained in Step C was dissolved in 10 ml EtOH and 5 ml cyclohexene containing 50 mg of 20% $Pd(OH)_2$ on carbon and 0.015 ml glacial acetic acid. The mixture was heated to reflux for 8 hours, then filtered through Celite® and stripped in vacuo. The resulting solid was recrystallized from CH_3OH to give 90 mg of product, 56% yield, mp 291-292°C. MS(NH_3): 794 (M+2, 60%); 793 (M+1, 100%).

Analysis for $C_{43}H_{64}N_6O_8 \bullet C_2H_4O_2$:

Calc'd:	C 63.36	H 8.03	N 9.85
Found:	63.28	8.28	9.98.

Example 12

N^α(1-Adamantanecarbonyl)Lys-Pro-Tyr-Ile-Leu(OMe)•HCl (SEQ ID NO:41)

100 mg of the acetic acid salt of the compound of SEQ ID NO:3 was dissolved in 3 ml of 4 M HCl/dioxane and 5 ml methanol then stirred for 2 hours at ambient temperature. The solvent was then removed in vacuo and the resulting residue was chromatographed using 30% methanol in chloroform as the eluting solvent. 100 mg (99%) of a colorless solid was isolated.

MS (calc'd)	MS (found)
808	808

Analysis

	Cal	Exp.
%C	62.56	62.20
%H	8.18	8.08
%N	9.95	9.80

m.p. 87-90°C.

Example 13

N^{α}(nicotinoyl)Lys-Pro-Tyr-Ile-Leu•HOAc (SEQ ID NO:43)

Step A: N^{α}(nicotinoyl)Lys-Pro-Tyr(OBzl)Ile-Leu(OBzl) (SEQ ID NO:42)

2.0 g (2.04 mmol) of the compound of Example 3, Step H was coupled with 0.36 g (2.04 mmol) of nicotinoyl chloride hydrochloride in the same manner as described above in Example 4, Step H. 1.39 (65% yield) of a colorless solid was isolated.

MS(calc'd)	MS(found)
1051	1051

Analysis

	Cal.(0.5 H_2O)	Exp.
%C	67.35	67.35
%H	7.02	6.97
%N	9.17	9.00.

Step B: N^{α}(nicotinoyl)Lys-Pro-Tyr-Ile-Leu•HOAc (SEC ID NO:43)

200 mg (0.19 mmol) of the product from Step A described above was deprotected according to the same procedure as that described above in Example 3, Step J to give 150 mg (99% yield) of a colorless solid.

MS(calc'd)	MS(found)
737	737

m.p. 68-70°C.

Example 14

N^{α}(Boc)Orn-Pro-ψ[CH_2NH]Tyr-Ile-Leu•HOAc (SEQ ID NO:45)

Step A: N^{α}(Boc)N^{δ}(Z)Orn-Pro-ψ[CH_2NH]Tyr(OBzl)-Ile-Leu-(OBzl) (SEQ ID NO:44)

1.0 g (1.4 mmol) of the compound in Example 7, Step C was coupled to N^{α}(Boc)N^{δ}(Z)Orn (0.52 g, 1.4 mmol) using the same procedure as described above in Example 3, Step G to give 0.32 g of a colorless solid.

MS(calc'd)	MS(found)
1018	1018

^{1}H NMR (300 MHz, DMSO/TMS δ): 8.4(d, 1H); 7.8(d, 1H); 7.4-7.3(m, 14H) 7.2(m, 1H) 7.1(m, 2H); 6.9-6.8(m, 3H); 5.1(s, 2H); 5.0(s, 2H); 4.95(s, 2H); 4.3(m, 1H); 4.2(t, 1H); 4.05(m, 1H); 3.9(m, 1H); 3.0(bs, 2H); 2.8(m, 1H); 2.2(m, 2H); 1.9-1.5(m, 10H); 1.4(s, 9H); 1.0(m, 1H); 0.9-0.7(m, 13H).

Step B: N^{α}(Boc)Orn-Pro-ψ[CH_2NH]Tyr-Ile-Leu•HOAc (SEQ ID NO:45)

0.14 g (0.14 mmol) of product obtained above in Example 14, Step A was deprotected using the same procedure as described above in Example 3, Step J to give 90 mg (84% yield) of product.

MS(calc'd)	MS(found)
704	704

R_f = 0.3 (20% methanol in chloroform).

Example 15

N^{α}(Boc)Orn-Pro-Tyr-ψ[CH_2NH]-Ile-Leu•HOAc (SEQ ID NO:49)

Step A: N^{α}(Boc)Tyr(Bzl)-ψ[CH_2NH]Ile-Leu(OBzl)

1.0 g (2.8 mmol) of N(Boc)Tyrosinol(Bzl), 2.8 g (28 mmol) triethylamine, and 4.46 g (28 mmol) of sulfur trioxide-pyridine complex were mixed with 25 ml DMSO and allowed to stir at ambient temperature for 15 min. The mixture was then poured into ice water and extracted (3 x 100 ml) with diethyl ether, dried and solvent removed _in vacuo_. 500 mg of the resulting oil was then coupled with 521 mg (1.41 mmol) of Ile-Leu(OBzl)•HCl using the same procedure as that described above for Example 8, Step B to give 0.5 of a colorless solid.

MS(calc'd)	MS(found)
673	673

^{1}H NMR (300 MHz, DMSO/TMS δ): 8.2(m, 1H); 7.4(m, 13H); 7.1(m, 2H); 6.9(m, 2H); 6.7(m, 1H); 5.1(s, 2H); 5.05(s, 2H); 4.4(m, 1H); 3.6(m, 1H); 2.7(m, 2H); 1.8(m, 1H); 1.6(m, 4H); 1.5(m, 2H); 1.3(s, 9H); 1.2(m, 4H); 0.9-0.8(m, 15H).

Step B: Tyr-(OBzl)-ψ[CH_2NH]-Ile-Leu(OBzl)•HCl

0.5 g (0.7 mmol) of the compound made in Step A above was deprotected in the same manner as that described above in Example 3, Step B to give 0.45 g (quantitative) of a crystalline solid.

MS(calc'd)	MS(found)
573	573

^{1}H NMR (300 MHz, DMSO/TMS δ): 8.6(bs, 2H); 7.4(m, 10H); 7.15(d, 2H); 6.95(d, 2H); 5.15(s, 2H); 5.05(s, 2H);

4.4(m, 1H); 3.7(m, 4H); 3.0(m, 2H); 2.0(m, 1H); 1.6(m, 3H); 1.1(m, 1H); 0.9(d, 2H); 0.9-0.8(m, 9H).

Step C: (Boc)Pro-Tyr(OBzl)-ψ[CH_2NH]-Ile-Leu(OBzl) (SEQ ID NO:46)

0.45 g (0.7 mmol) of the compound made in Example 15, Step B was coupled to Boc-L-proline (177 mg, 0.82 mmol) in the same manner as that described above in Example 3, Step B to give 0.47 g (87% yield) of a colorless solid.

MS(calc'd)	MS(found)
770	770

R_f = 0.5 1:1 EtOAc:Hexane.

Step D: Pro-Tyr(OBzl)-ψ[CH_2NH]-Ile-Leu(OBzl)•HCl (SEQ ID NO:47)

0.47 g of the compound made in Example 15, Step C was deprotected in the same manner as that described for Example 3, Step B to give 0.4 g of a crystalline solid.

MS(calc'd)	MS(found)
670	670

^{1}H NMR (300 MHz, DMSO/TMS δ): 9.65(m); 9.2(m, 2H); 9.0(m, 2H); 8.6(m); 7.4(m, 10H); 7.1(d, 2H, J=8.5HZ); 6.9(d, 2H, J=8.4HZ); 5.15(s, 2H); 5.05(s, 2H); 4.4(m, 1H); 4.1(m, 2H; 3.8(m, 1H); 3.2(m, 2H); 3.1(m, 1H); 2.8(m, 3H); 2.2(m, 1H); 2.0(m, 2H); 1.9(m, 2H); 1.6(m, 4H); 1.1(m, 1H); 0.9-0.8(m, 11H).

Step E: N^{α}(Boc),N^{δ}(Z) Orn-Pro-Tyr(OBzl)-ψ[CH_2NH]-Ile-Leu(OBzl) (SEQ ID NO:48)

0.2 g (0.28 mmol) of the compound made in Example 15, Step D was coupled to 0.103 g (0.28 mmol) of N^{α}(Boc) N^{δ}(Z)Orn in the same manner as that described above in Example 3, Step G to give 0.1 g (35% yield) of a colorless solid.

MS(calc'd)	MS(found)
1017	1017

R_f = 0.4 1:1 EtOAc:Hexane.

Step F: N^{α}(Boc)-Orn-Pro-Tyrψ[CH_2NH]-Ile-Leu•HOAc (SEQ ID NO: 49)

0.1 g of the compound made in Example 15, Step E was deprotected in the same manner as that described in Example 3, Step J to give 0.08 g of a white crystalline solid.

FAB-MS (calc'd)	FAB-MS (found)	
705.45	705.52	$(M+H)^+$
727.44	727.50	$(M+Na)^+$.

The compounds described below in Examples 16-20 were prepared using the synthetic procedure described above in Example 3 by selecting the appropriate acid chloride.

Example 16

N^{α}-(PhCO)-Lys-Pro-Tyr-Ile-Leu•HOAc (SEQ ID NO:50)

MS-DCI (NH_3): 737 (M+H, base)

$[\alpha]_D^{25}$ -54.1° (c = 0.616, MeOH)

Anal. Calc'd. for $C_{41}H_{60}N_6O_{10}$•1.5 H_2O:

	C 59.76	H 7.71	H 10.20
Found:	59.56	7.77	10.20.

Example 17

N^{α}-(t-BuCO)-Lys-Pro-Tyr-Ile-Leu•HOAc (SEQ ID NO:51)

MS-DCI (NH_3): 717 (M+H, base)

$[\alpha]_D^{25}$ -66.8° (c = 0.608, MeOH)

Anal. Calc'd. for $C_{39}H_{64}N_6O_{10}$•2 H_2O:

	C 57.62	H 8.43	H 10.34
Found:	57.74	8.36	10.21.

Example 18

N^{α}-(t-$BuCH_2CO$)-Lys-Pro-Tyr-Ile-Leu•HOAc (SEQ ID NO:52)

MS-DCI (NH_3): 731 (M+H, base)

R_f (chloroform/methanol/benzene/water 8:8:8:1) = 0.31.

Example 19

N^{α}-(4-Ph-C_6H_4-CO)-Lys-Pro-Tyr-Ile-Leu•HOAc (SEQ ID NO:53)

MS-DCI (NH_3): 813 (M+H, base)

$[\alpha]_D^{25}$ -48.9° (c = 0.608, MeOH)

R_f (chloroform/methanol/benzene/water 8:8:8:1) = 0.37.

Example 20

N^{α}-(4-t-Bu-C_6H_4-CO)-Lys-Pro-Tyr-Ile-Leu•HOAc (SEQ ID NO:54)

MS-DCI (NH_3): 793 (M+H, base)

$[\alpha]_D^{25}$ -52.9° (c = 0.612, MeOH)

Anal. Calc'd. for $C_{45}H_{68}N_6O_{10}$:

	C 63.36	H 8.03	H 9.85
Found:	63.18	8.29	9.89.

Example 21

N^{α}-(1-Adamantanecarbonyl)-Lys-ProΨ[CH=CH]-Tyr-Ile-Leu (SEQ ID NO:55)

This compound can be prepared according to the procedure described below in Example 34.

Example 22

N^{α}[$CH_3(CH_2)_{16}CO$]Lys-Pro-Tyr-Ile-Leu, Acetic Acid Salt (SEQ ID NO:57)

Example 22 was prepared according to the synthetic procedure described above in Example 3 by using the appropriate acid chloride.
MS-DCI (NH_3): 898 (M+H, Base)
R_f (chloroform/methanol 20:1)=0.40.

Example 23

N^{α}(1-adamantanecarbonyl)Lys-ψ[CH_2N]Pro-Tyr-Ile-Leu, Acetic Acid Salt (SEQ ID NO:58)

Step A: N^{α}BocN^{ε}(Z)Lysinal

N^{α}BocN^{ε}(Z)-Lysinal was prepared from the corresponding alcohol in the same manner as described above in Example 7, Step A.
MS-DCI (NH_3) 364 $(M+H)^+$ and 382 $(M+NH_4)^+$
^{1}H NMR (300 MHz, DMSO/TMS δ) 7.4(m, 6H); 7.0(m, 1H); 5.0(s, 2H); 3.7(d, 2H); 3.0(m, 3H); 2.4(m, 3H); 1.4(s, 15H).

Step B: N^{α}BocN^{ε}(Z)-Lysψ[CH_2N]Pro-Tyr(OBzl)Ile-Leu(OBzl) (SEQ ID NO:59).

The aldehyde from Step A was coupled to the product from Example 3, Step F using the synthetic procedure described in Example 7, Step B.
MS-DCI (NH_3) 1033 (M+H, Base)
R_f (Ethylacetate/hexane 1.7:1)=0.50.

Step C: N^{ε}(Z)-Lysψ[CH_2N]Pro-Tyr(OBzl)Ile-Leu(OBzl), Hydrochloride Salt (SEQ ID NO:60)

The product from Step B above was deprotected according to the procedure described above in Example 3, Step B.

MS-DCI 933 (M+H), Base)

R_f (chloroform/methanol 20:1)=0.32.

Step D: N^{α}(1-Adamantanecarbonyl)N^{ε}(Z)-Lysψ[CH_2N]Pro-Tyr(OBzl)Ile-Leu(OBzl) (SEQ ID NO:61)

The product from Step C above was coupled to 1-adamantane carbonyl chloride in the same manner as described above in Example 3, Step I.

MS-DCl (NH_3) 1095 (M+H, Base)

R_f (chloroform/methanol 20:1)=0.5.

Step E: N^{α}(1-Adamantanecarbonyl)Lysψ[CH_2N]Pro-Tyr-Ile-Leu, Acetic Acid Salt (SEQ ID NO:58)

The product from Step D above was deprotected in the same manner as described above in Example 3, Step J.

	Measured	Calculated
FAB MS	781.57	781.52 (M+H)
	803.56	803.50 (M+Na).

Example 24

N^{α}(1-Adamantanecarbonyl)Lys-Pro-Tyrψ[CH_2NH]Ile-Leu, Acetic Acid Salt (SEQ ID NO:62)

Step A: N^{α}(Boc)N^{ε}(Z)-Lys-Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:63)

N^{α}(Boc)N^{ε}Lys was coupled with the product of Example 15, Step D above in the same manner described above for Example 3, Step G.

MS - DCI (NH_3) 1033 (M+H)

R_f (chloroform/methanol 20:1)=0.82.

Step B: N^{ε}(Z)Lys-Pro-Tyr(OBzl)ψ[CH_2NH]Ile-Leu(OBzl) Hydrochloride Salt (SEQ ID NO:64)

The compound from Step A above was deprotected using the same conditions as that described above for Example 3, Step B.
MS - DCI (NH_3) 933 (M+H, Base)
R_f (chloroform/methanol 20:1)=0.30.

Step C: N^{α}(1-Adamantanecarbonyl)N^{ε}(Z)Lys-Pro-Tyr(OBzl)ψ[CH_2NH]Ile-Leu(OBzl) (SEQ ID NO:65)

The product from Step B above was coupled with 1-adamantane carbonyl chloride in the same manner as that described above for Example 3, Step I.
MS-DCI (NH_3) 1095 (M+H)
R_f (chloroform/methanol 20:1) = 0.8.

Step D: N^{α}(1-Adamantanecarbonyl)Lys-Pro-Tyrψ[CH_2NH]Ile-Leu, Acetic Acid Salt (SEQ ID NO:62)

The product from Step C above was deprotected in the same manner as that described above for Example 3, Step J.

	Measured	Calculated
FAB MS	781.52	781.52 (M+H)

R_f (chloroform/methanol 20:2)=0.3.

Example 25

The following peptides were prepared according to the procedure described above in Example 1.

Sequence	SEQ ID NO	FAB-MS Calc'd	(M+H) Found
H-Nle-Arg-Pro-Tyr-Ile-Leu	66	774.49	774.09
H-Arg-Nle-Pro-Tyr-Ile-Leu	67	774.49	774.55
pGlu-Arg-Pro-Tyr-Ile-Leu	68	772.57	772.44
Ada-Lys-Pro-Pro-Tyr-Ile-Leu	69	892.55	892.71
Ada-Arg-Arg-Pro-Tyr-Tle-Leu	70	979.61	979.73
H-Cha(4-NH_2)-Pro-Pro-Tyr-Ile-Leu	71	770.48	770.57
H-Cha(4-NH_2)-Arg-Pro-Tyr-Ile-Leu	72	829.53	829.51
H-Pro-Arg-Pro-Tyr-Ile-Leu	73	758.46	758.47
H-Arg-Cha(4-NH_2)-Pro-Tyr-Ile-Leu	74	829.53	829.57
Ada-Lys-Pro-Tyr-Tle-Leu	75	795.50	795.60
Fmoc-Lys-Pro-Tyr-Ile-Leu	76	855.47	855.45
H-Arg-(Me)Nle-Pro-Tyr-Ile-Leu	77	788.50	788.47
N^{α}-acetyl-Arg-Arg-Pro-Tyr-Pgl-Leu	38	879.48	879.56

Example 26

N-t-Boc-Ala-Pro-Tyr-Ile-Leu • 1/2 H_2O

(SEQ ID NO:78)

Step A: N-Boc-Ala-Pro-Tyr(OBzl)-Ile-Leu(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:79)

N-Boc-Ala (0.95 g) was coupled using the procedure described above for Example 3, Step A to Pro-Tyr(OBzl)-Ile-Leu(OBzl)•HCl (3.6 g) [SEQ ID NO:10] to yield 1.9 g (44% yield) of N-Boc-Ala-Pro-Tyr-(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:79)•1/2 H_2O as a colorless solid.

MS(calc'd)	MS(found)	
856	856	$(M+H)^+$

Analysis

	Cal	Exp.
%C	67.35	67.01
%H	7.65	7.74
%N	8.18	8.03

$[\alpha]_D^{25}$ -59.7° (c = 0.610, CHCl3).

Step B: N-t-Boc-Ala-Pro-Tyr-Ile-Leu (SEQ ID NO:78)

N-Boc-Ala-Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:79) (1.2 g) was deprotected using the method of Example 3, Step J (except no acetic acid was added) to yield 1.1 g of N-t-Boc-Ala-Pro-Tyr-Ile-Leu•1/2 H_2O (SEQ ID NO:78) as a white solid.

MS(calc'd)	MS(found)	
676	676	$(M+H)^+$

Analysis

	Cal	Exp.
%C	59.63	59.51
%H	7.95	8.13
%N	10.23	9.88

$[\alpha]_D^{25}$ -79.4° (c = 0.514, MeOH).

Example 27

6-Aminocaproyl-Pro-Tyr-Ile-Leu Hydrochloride

(SEQ ID NO:80)

Prepared according to the procedure described above in Example 3.

MS(calc'd)	MS(found)	
618	618	$(M+H)^+$

$[\alpha]_D^{25}$ -63.90° (c = 0.498, H_2O).

Example 28

N^α-Boc-Lys-Pro-Tyr-L-phenylglycyl-Leu•H_2O

(SEQ ID NO:81)

Prepared according to the procedure described above in Example 3.

MS(calc'd)	MS(found)	
753	753	$(M+H)^+$

	Analysis	
	Cal	Exp.
%C	58.00	58.13
%H	7.60	7.40
%N	9.90	9.79

$[\alpha]_D^{25}$ -32.9° (c = 0.474, MeOH).

Example 29

N$^{\alpha}$-Boc-Lys-Pro-Trp-Ile-Leu•HOAc•H_2O (SEQ ID NO:82)

This compound was prepared according to the procedure described above in Example 3.

MS(calc'd)	MS(found)	
756	756	$(M+H)^+$

	Analysis	
	Cal	Exp.
%C	59.05	59.03
%H	8.10	8.12
%N	11.76	11.51

$[\alpha]_D^{25}$ -60.6° (c = 0.630, MeOH).

Example 30

N-[trans-4-(aminomethyl)cyclohexane carbonyl]-Pro-Tyr-Ile-Leu•HOAc•H_2O (SEQ ID NO:83)

This compound was prepared according to the procedure described above in Example 3.

MS(calc'd)	MS(found)	
644	644	$(M+H)^+$

	Analysis	
	Cal	Exp.
%C	59.90	59.73
%H	8.24	8.11
%N	9.70	9.38

$[\alpha]_D^{25}$ -48.4° (c = 0.612, MeOH).

Example 31

N-[2-(aminomethyl)benzoyl]-Pro-Tyr-Ile-Leu•HCl (SEQ ID NO:84)

This compound was prepared according to the procedure described above in Example 3.

MS(calc'd)	MS(found)	
638	638	$(M+H)^+$
620	620	$(M+H-H_2O)^+$
	505	
	168	base peak
	151	

$[\alpha]_D^{25}$-92.1° (c = 0.604, MeOH)
TLC R_f (n-BuOH/EtOAc/H_2O/HOAc 1:1:1:1) = 0.59.

Example 32

N-[3-(aminomethyl)benzoyl]-Pro-Tyr-Ile-Leu•HCl (SEQ ID NO:85)

This compound was prepared according to the procedure described above in Example 3.

	MS(calc'd)	MS(found)	
	638	638	$(M+H)^+$

$[\alpha]_D^{25}$ -53.5° (c = 0.602, MeOH)
TLC R_f ($CHCl_3$/MeOH/PhH/H_2O 8:8:8:1) = 0.20.

Example 33

N-[(4-aminomethyl)benzoyl]-Pro-Tyr-Ile-Leu•HCl (SEQ ID NO:86)

This compound was prepared according to the procedure described above in Example 3.

	MS(calc'd)	MS(found)	
	638	638	$(M+H)^+$

$[\alpha]_D^{25}$ -32.4° (c = 0.602, DMSO)
TLC R_f (n-BuOH/EtOAc/HOAc/H_2O 1:1:1:1) = 0.59.

Example 34

N^{α}-Boc-Lys-Proψ[trans-CH=CH]Tyr-Ile-Leu (SEQ ID NO:87)

Step A: 1-tert-Butoxycarbonyl-2-(S)-propenonepyrrolidine

In a 1-L three-neck flask equipped with a magnetic stirrer, 6 g (27.88 mmoles) Boc-Proline was dissolved in 400 ml dry THF and cooled to -78°C. 17 ml of 1.6 M solution nBuLi in hexanes (27.2 mmoles) was added to the mixture followed by 60 ml (60 mmoles) vinylmagnesium bromide, 1 M solution in THF (60 mmoles). Then it was allowed to warm to 25°C, and stirred for 3 hours. Then 30 ml vinylmagnesium bromide was added and stirred for 1 hour, quenched with 300 ml 10% HCl solution (1 M) and extracted with EtOAc (3x300 ml). The EtOAc was washed with 200 ml 5% NaHCO3 and brine, dried and stripped *in*

vacuo. The resulting oil, 4.55 g, was used for the next reaction without purification.
NMR ($CDCl_3$)δ: 6.3-6.6(m, 2H); 5.8-5.85(m, 1H); 4.6-4.7 and 4.4-4.5(m m, 1H); 3.4-3.6(m, 2H); 2.1-2.3(m, 1H); 1.8-1.95(m, 3H); 1.45 and 1.35(s s, 9H).
MS(NH_3) m/e: 226 (23%, M+1); 187 (51%, M-C_4H_8+18).

Step B: 1-tert-Butoxycarbonyl-2-(S)-(1'-(S)-hydroxyprop-2-ene)pyrrolidine

In a 200 ml flask 4.55 g (20.2 mmoles) of 1-tert-butoxycarbonyl-2-(S)-propenonepyrrolidine was dissolved in 100 ml MeOH, 6.6 g (18 mmoles) $CeCl_3 \cdot 7H_2O$ was added, and cooled to -78°C. Then 680 mg (17.8 mmoles) $NaBH_4$ was added, stirred at -78°C for 4 hours, allowed to warm to -10°C and quenched with 50 ml 10% HCl. The mixture was extracted with EtOAc (3x100 ml) and the organic extracts washed with 80 ml 5% $NaHCO_3$ and brine, dried and stripped *in vacuo*. The remaining was chromatographed on silica gel using 20% EtOAc/Hexanes as eluent to give 3.8 g of product, a 36% yield for the two steps.
NMR ($CDCl_3$)δ: 5.65-5.9(m, 1H); 5.15-5.35(m, 3H); 3.8-4.2(m, 2H); 3.1-3.55(m, 2H); 1.65-1.95(m, 4H); 1.45(s, 9H). MS(NH_3) m/e: 228 (100%, M+1); 172 (82%, M-C_4H_8).

Step C: 1-tert-Butoxycarbonyl-2-(S)-(1'-(S)-O(-2''-hexahydropyrane)prop-2-ene)pyrrolidine

In a 300 ml flask 3.8 g 16.72 (mmoles) 1-tert-butoxycarbonyl-2-(S)-(1'-(S)-hydroxyprop-2-ene)pyrrolidine was dissolved in 90 ml dry CH_2Cl_2 containing 2.7 ml (29.7 mmoles) tetrahydropyrane and 50 mg (2 mmoles) pyridinium paratoluenesulfonate. After the mixture was stirred at 25°C for 3 hours it was poured into a separatory funnel containing 600 ml EtOAc and 200 ml 5% $NaHCO_3$. The EtOAc was washed with brine, dried and stripped *in vacuo*. The remaining was

chromatographed on silica gel using 10% EtOAc/Hexanes to give 4.3 g product, an 83% yield.
NMR ($CDCl_3$)δ: 5.6-5.9(m, 1H); 5.1-5.4(m, 2H); 4.5-4.8(m, 2H); 3.2-4.2(m, 5H); 2.1-1.4(m, 19H).
MS (NH_3) m/e: 312 (39%, M+1); 228 (100%, M-C_5H_8O).

Step D: 1-tert-Butoxycarbonyl-2-(S)-(1'-(S)-O(-2''-hexahydropyrane)2-carboxaldehyde)pyrrolidine

In a 500 ml flask 4 g (12.85 mmoles)1-tert-butoxycarbonyl-2-(S)-(1'-(S)-O(-2''-hexahydro-pyrane)prop-2-ene)pyrrolidine was dissolved in 300 ml CH_2Cl_2 containing 2.88 ml pyridine and cooled to -78°C. To that O_3 was passed through until it turned pale blue. The excess O_3 was removed with oxygen, 4 ml methyl sulfide was added, and the mixture was allowed to warm to 25°C and stirred for 3 hours. The solvent was stripped in vacuo and the resulting oil was used directly for the next reaction.

Step E: 1-tert-Butoxycarbonyl-2-(S)-(1'-(S)-O(-2''-hexahydropyrane)4'-carbomethoxybut-2'-ene)pyrrolidine

In a 300 ml flask 834 mg (19.2 mmoles) of 60% NaH in oil was washed with 60 ml hexanes, suspended in 75 ml THF, and cooled to 0°C. In this 3.4 ml (21 mmoles), $(CH_3O)_2P(O)CH_2CO_2CH_3$ was added and the mixture stirred for 45 minutes. Then it was cooled down to -78°C and 6 g (19.2 mmoles) of 1-tert-butoxycarbonyl-2-(S)-(1'-(S)-O(-2''-hexahydropyrane)2-carboxaldehyde)pyrrolidine in 40 ml THF was added, the reaction was stirred at -78°C for 1 hour and at 0°C for 2 hours. Then it was poured into a separatory funnel containing 500 ml EtOAc and 200 ml 10% HCl. The organic layer was washed with 100 ml 5% $NaHCO_3$ and brine, dried, and stripped in vacuo. The remaining oil was chromatographed on silica gel using

10% EtOAc/Hexanes to give 4 g of product, a 56% yield for the two steps.
NMR ($CDCl_3$)δ: 6.75-6.95(m, 1H); 5.7-6.2(m, 1H); 4.55-5.0(m, 2H); 3.7-4.0(m, 2H); 3.75(s, 3H); 3.2-3.4(m, 4H); 1.4-2.2(m, 19H). MS (NH_3) m/e: 370 (13%, M+1); 286 (100%, M-C_5H_8O).

Step F: 1-tert-Butoxycarbonyl-2-(S)-(1'-(S)-hydroxy-4'-carbomethoxybut-2'-ene)pyrrolidine

In a 100 flask 2.86 g (7.72 mmoles) of 1-tert-butoxycarbonyl-2-(S)-(1'-(S)-O(-2"-hexahydropyrane)4'-carbomethoxybut-2'-ene)pyrrolidine was dissolved in 50 ml MeOH containing 150 mg (0.66 mmoles) camphorsulfonic acid. The reaction was stirred at 25°C for 3 hours, quenched with 5 ml 5% $NaHCO_3$, 200 mg $NaHCO_3$ and stripped <u>in vacuo</u>. The remaining was dissolved in 200 ml EtOAc and washed with 30 ml water, and brine, dried and the solvent was stripped <u>in vacuo</u>. The product was purified by silica gel chromatography to give 1.2 g of product, a 54% yield.
NMR ($CDCl_3$)δ: 6.85-7.0(m, 1H); 6.1-6.25(m, 1H); 5.45-5.6(m, 1H); 3.8-4.4(m, 2H); 3.73(s, 3H); 3.15-3.5(m, 2H); 1.65-2.2(m, 4H); 1.23, 1.27(s s 9H). MS (NH_3) m/e: 286 (35%, M+1); 247 (100%, M-C_4H_8+18); 230 (50%, M-C_4H_8).

Step G: 1-tert-Butoxycarbonyl-2(S)(1'-(S)-O-methane-sulfonyl-4'-carbomethoxybut-2'-ene)pyrrolidine

In a 35 ml 790 mg (2.77 mmoles)1-tert-butoxycarbonyl-2-(S)-(1'-(S)-hydroxy-4'-carbomethoxybut-2'-ene)pyrrolidine, dissolved in 10 ml CH_2Cl_2 was cooled to 0°C, was treated with 0.57 ml (3.27 mmoles)diiso-propylethyl amine followed by 0.25 ml (3.27 mmoles) methanesulfonyl chloride. After stirring at 0°C for 2 hours the reaction was poured into a separatory funnel containing 300 ml EtOAc and 50 ml water. The EtOAc was

washed with 10% HCl, 5% $NaHCO_3$ and brine (50 ml each), dried over $MgSO_4$ and stripped in vacuo. The remaining was chromatographed on silica gel using 1% $MeOH/CH_2Cl_2$ as eluent to give 800 mg of product, an 80% yield. NMR ($CDCl_3$) δ: 6.83-6.93(m, 1H); 6.1-6.22(m, 1H); 5.6-5.83(m, 1H); 3.9-4.22(m, 1H); 3.78(s, 3H); 3.25-3.6(m, 2H); 2.98, 3.04-3.1(s m, 3H); 1.7-2.1(m, 4H); 1.48, 1.51 (s s, 9H). MS (NH3) m/e: 381 (16%, M+18); 364 (5%, M+1); 325 (100%, $M-C_4H_8+18$).

Step H: Methyl trans-2-(S)-Benzyloxybenzyl-4-(1'-tert-butoxycarbonyl-2'(S)-pyrrolidine)buten-3-ate

In a 20 ml flask 1.53 g (6.5 mmoles) benzyloxybenzyl chloride was treated with 400 mg in 10 ml THF at 0°C for 3 hours. Then it was transferred via cannula into a flask containing 486 mg (6.5 mmoles) CuCN suspended in 10 ml THF and cooled to -40°C in a CH_3CN/dry ice bath. The resulting mixture was stirred at -40°C for 1 hour and the 950 mg (2.6 mmoles) 1-tert-butoxycarbonyl-2(1'-O-methanesulfonyl-4'-carbomethoxybut-2'-ene)pyrrolidine in 5 ml THF was added. The resulting mixture was stirred at -40°C for 1 hour and allowed to warm up to 25°C and poured into a flask containing 50 ml of a 9:1 mixture of satNH_4Cl/concNH_4OH under vigorous stirring. After stirring for 30 minutes the product was extracted with EtOAc (2x50 ml), the EtOAc was washed with water and brine, dried over $MgSO_4$ and stripped in vacuo. The resulting oil was chromatographed on silica gel using 15% EtOAc/Hexanes as eluent to give 700 mg of product, a 58% yield.
NMR ($CDCl_3$) δ: 7.3-7.45(m, 5H); 7.4(d, 2H, J=7.5HZ); 6.86(d, 2H, J=7.5HZ); 5.42-5.57(m, 1H); 5.38(d d, 1H, J_1=15HZ, J_2=7.5HZ); 5.02(s, 2H); 4.1-4.4(m, 1H); 3.6(s, 3H); 3.2-3.4(m, 2H); 3.0(m, 1H); 2.72(m, 1H); 1.5-2.0(m,

4H); 1.4, 1.45(s s, 9H). MS (NH_3) m/e: 483 (22%, M+18); 466 (6%, M+1); 427 (100%, M-C_4H_8+18).

Step I: trans-2-(S)-Benzyloxybenzyl-4-(1'-tert-butoxycarbonyl-2'(S)-pyrrolidine)buten-3-oic acid

In a 25 ml flask 280 mg (0.59 mmoles) methyl trans-2-(S)-benzyloxybenzyl-4-(1'-tert-butoxycarbonyl-2'(S)-pyrrolidine)but-3-enoate was treated with 3 ml 0.2 M LiOH (0.6 mmoles) in 3 ml dioxane at 25°C for 16 hours. The reaction was then acidified with 10% $KHSO_4$ and extracted with 100 ml EtOAc, the EtOAc was washed with brine, dried over $MgSO_4$, and stripped <u>in vacuo</u> to give 250 mg of the acid, a 92% yield.

Step J: trans-2-(S)-Benzyloxybenzyl-4-(1'-tert-butoxycarbonyl-2'-(S)-pyrrolidine)buten-3-ate-Ile-Leu(OBzl) (SEQ ID NO:102)

In a 35 ml flask 250 mg (0.56 mmoles) of trans-2-(S)-benzyloxybenzyl-4-(1'-tert-butoxycarbonyl-2'(S)-pyrrolidine)but-3-enoic acid, in 6 ml THF was cooled to -10°C and 0.078 ml (0.69 mmoles) N-methylmorpholine, followed by 0.09 ml (0.69 mmoles) isobutyl chloroformate was added. The mixture was stirred at -10°C for 15 minutes and then a solution of 250 mg (0.69 mmoles) HCl•Ile-Leu(OBzl) in 3 ml DMF containing 0.076 ml (0.69 mmoles) N-methylmorpholine at -10°C was added via cannula. The reaction was stirred at -10°C for 1 hour and then allowed to warm to 25°C and stirred for 30 minutes and poured into a separatory funnel with 100 ml EtOAc and 20 ml 10% HCl. The EtOAc was washed with water and brine, dried over $MgSO_4$ and stripped <u>in vacuo</u>. The resulting solid was chromatographed on silica gel using 30% EtOAc/Hexanes as eluent to give 300 mg of product, a 70% yield.

NMR ($CDCl_3$)δ: 7.3-7.45(m, 10H); 7.07(d, 2H, J=8HZ); 6.85(d d, 2H, J=8HZ); 5.35-5.6(m, 2H); 5.15(d d, 2H); 5.02(s, 2H); 4.63(m, 1H); 4.2-4.3(m, 2H); 3.33(m, 2H); 3.0-3.15(m, 2H); 2.64-2.9(m, 1H); 1.0-2.0(m, 10H); 1.2(s, 9H); 0.85-1.0(m, 12H). MS (NH_3) m/e: 785 (100%, $M+NH_4$).

Step K: trans-2-(S)-Benzyloxybenzyl-4-(2'-(S)-pyrrolidine)buten-3-ate-Ile-Leu(OBzl) hydrochloride (SEQ ID NO:103)

In a 35 ml flask 170 mg (0.22 mmoles) trans-2-(S)-benzyloxybenzyl-4-(1'-tert-butoxycarbonyl-2'(S)-pyrrolidine)buten-3-ate-Ile-Leu(OBzl) (SEQ ID NO:102) was dissolved in 2 ml CH_2Cl_2 and 0.5 ml 4.5 M HCl solution in dioxane was added. After stirring at 25°C for 3 hours the solvent was stripped <u>in vacuo</u> and the hydrochloride was precipitated from ether, filtered and dried to give 120 mg of product which was used for the next reaction without purification.

Step L: (N^{α}Boc)(N^{ϵ}CBZ)Lys-trans-2-(S)-4'-Benzyloxybenzyl-4-(2'-(S)-pyrrolidine)buten-3-ate-Ile-Leu(OBzl) (SEQ ID NO:104)

trans-2-(S)-Benzyloxybenzyl-4-(2'-(S)-pyrrolidine)buten-3-ate-Ile-Leu(OBzl) (SEQ ID NO:103) hydrochloride, 120 mg (0.17 mmoles) was treated with 65 mg (0.17 mmoles (N^{α}Boc)(N^{ϵ}CBZ)Lysine, 35 mg (0.17 mmoles) DCC, 26 mg (0.17 mmoles) 1-hydroxybenzotriazole and 0.022 ml N-methylmorpholine in 1 ml DMF at 25°C for 20 hours. Then it was poured into a separatory funnel containing 100 ml EtOAc and 20 ml 10% HCl. The EtOAc was washed with 5% $NaHCO_3$, and brine, dried, and stripped <u>in vacuo</u>. The resulting solid was purified by prep. plate chromatography to give 110 mg of product, a 48% yield for the two steps.

NMR ($CDCl_3$)δ: 7.25-7.45(m, 15H); 7.0-7.1(m, 2H); 6.8-6.9(m, 2H); 6.5(m, 1H); 6.32(m, 1H); 5.2-5.55(m, 4H); 5.15(s, 2H); 5.0-5.15(m, 2H); 5.02(d, 2H); 4.0-4.7(m, 4H); 2.6-3.8(m, 7H); 1.1-2.0(m, 25H); 0.7-1.0(m, 12H). MS (NH_3) m/e: 1047 (100%, M+NH_4).

Step M: N^{α}-Boc-Lys-Proψ[trans-CH=CH]Tyr-Ile-Leu (SEQ ID NO:87)

N^{α}-Boc(N^{ϵ}CBZ)Lys-trans-2-(S)-4'-Benzyloxybenzyl-4-(2'-(S)-pyrrolidine)but-3-enoate-Ile-Leu(OBzl) (SEQ ID NO:103), 110 mg (0.11 mmoles) was dissolved in 6 ml ethanol, 3 ml cyclohexane and 0.010 ml acetic acid and 30 mg 20% $Pd(OH)_2/C$ was added. The mixture was heated to reflux for 3.5 hours and then filtered through Celite® and stripped <u>in vacuo</u>. The resulting solid was chromatographed on silica gel using 1% NH_4OH, 10% $MeOH/CH_2Cl_2$, followed by 2% NH_4OH, 20% MeOH and 3% NH_4OH, 30% $MeOH/CH_2Cl_2$, to give 40 mg of product, a 52% yield, mp 150-152°C.
NMR (CD_3OD)δ: 6.98(m, 3H); 6.62-6.72(m, 3H); 5.6(m, 1H); 5.45(m, 1H); 5.32(m, 1H); 3.95-4.6(m, 4H); 2.8-3.1(m, 6H); 2.6-2.7(m, 1H); 1.0-2.0(m, 25H); 0.7-1.0(m, 12H). MS(NH_3) m/e: 716 (100%, M+1).

Example 35

2-Benzyl-5-Aminopentanecarbonyl-Pro-Tyr-Ile-Leu (SEQ ID NO: 88)

Step A: 5-Di(p-methoxybenzyl)aminovaleric acid

In a 500 ml flask 5.85 g (50 mmoles) 5-aminovaleric acid was dissolved in 260 ml methanol and 13.8 g (100 mmoles) $ZnCl_2$ was added. The mixture was cooled to 0°C and 6.2 g (100 mmoles) $NaCNBH_3$ was added, followed by 13.8 g (100 mmoles) p-anisaldehyde. The mixture was allowed to warm to 25°C and stirred at that temperature for 20 hours. The methanol was stripped off under

vacuum, and the remaining solid was partitioned between 300 ml CH_2Cl_2 and 300 ml water. The water was extracted with 100 ml CH_2Cl_2, and the combined CH_2Cl_2 extracts washed with water and brine, dried and stripped *in vacuo*, to give 19 g of crude product, which was used directly for the next reaction.
NMR ($CDCl_3$)δ: 7.36(d, 4H, J=8HZ); 6.9(d, 4H, J=8HZ); 3.94(s, 4H); 3.8(s, 6H); 2.8(m, 2H); 2.3(m, 2H); 1.78(m, 2H); 1.55(m, 2H).

Step B: Ethyl 5-di(p-methoxybenzyl)amino valerate

In a 500 ml flask 19 g crude 5-di(p-methoxybenzyl)aminovaleric acid was dissolved in 300 ml ethanol and 3 ml conc. H_2SO_4 was added. The mixture was heated to reflux for 5 hours, and then stirred at 25°C for 16 hours. The acid was neutralized with solid $NaHCO_3$ and the ethanol was stripped off. The resulting oil was extracted with EtOAc (2x150 ml), and the combined EtOAc extracts were washed with 60 ml 5% $NaHCO_3$ and brine, dried, and stripped *in vacuo*. The resulting oil was chromatographed on silica gel using 10% EtOAc/Hexanes as eluent, to give 12 g of product, a 62% yield for the two steps.
NMR ($CDCl_3$)δ: 7.24(d, 4H, J=7.5HZ); 6.84(d, 4H, J=7.5HZ); 4.1(q, 2H), J=7HZ); 3.8(s, 6H); 3.45(s, 4H); 2.38(t, 2H); 2.2(t, 2H); 1.5-1.65(m, 4H); 1.23(5, 3H, J=7HZ).

Step C: Ethyl 2-benzyl-5-di(p-methoxybenzyl)amino valerate

In a 35 ml flask 0.65 ml (4.72 mmoles) diisopropylamine was dissolved in 10 ml dry THF and cooled to -78°C in a dry ice/acetone bath. To that 2.9 ml (4.7 mmoles) of a 1.6 M solution nBuLi in hexanes was added, the resulting mixture was allowed to warm to

25°C, cooled again to -78°C, and a solution of 1.5 g (3.92 mmoles) ethyl 5-di(p-methoxybenzyl)amino valerate in 5 ml dry THF was added. The reaction was stirred at -78°C for 40 minutes, 0.6 ml (4.7 mmoles) benzyl bromide was added and the resulting mixture was stirred at -78°C for 1.5 hours. Then it was allowed to warm to 25°C and poured into a separatory funnel containing 100 ml EtOAc and 20 ml water, the water was extracted with 50 ml EtOAc and the combined EtOAc was washed with brine, dried, and stripped in vacuo. The resulting oil was chromatographed on silica gel using 18% EtOAc/Hexanes as eluent to give 1.51 g product, an 82% yield.

NMR ($CDCl_3$)δ: 7.2-7.3(m, 7H); 7.1(m, 1H); 6.84(d, 4H, J=8HZ); 4.03(q, 2H, J=7HZ); 3.8(s, 6H); 3.22(dd, 4H, J=14HZ); 2.9(dd, 1H, J_1=14HZ, J_2=7HZ); 2.7(dd, 1H, J_1=14HZ, J_2=7HZ); 2.56(m, 1H); 2.35(m, 1H); 1.5(m, 4H); 1.1(t, 3H, J=7HZ).

Step D: 2-Benzyl-5-di(p-methoxybenzyl)amino valeric acid

In a 30 ml flask 760 mg (1.6 mmoles) ethyl 2-benzyl-5-di(p-methoxybenzyl)amino valerate was dissolved in 8 ml methanol and 4 ml 1 M LiOH was added. The mixture was heated to reflux for 8 hours, neutralized with 10% HCl, and extracted with 100 ml CH_2Cl_2. The CH_2Cl_2 extract was washed with brine, dried and stripped in vacuo, to give 700 mg of the acid which was used without purification.

NMR ($CDCl_3$)δ: 7.05-7.3(m, 9H); 6.83(d, 4H, J=8HZ); 3.8(s, 6H); 3.8(d, 2H, J=12HZ); 3.05(dd, 1H, J_1=14HZ, J_2=7HZ); 2.4-2.65(m, 4H); 1.75(m, 2H); 1.55(m, 1H); 1.35(m, 1H).

Step E: 2-Benzyl-4-di(p-methoxybenzyl)aminopentane-carbonyl-Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:89)

In a 25 ml flask 820 mg (1.14 mmoles) HCl•Pro-Tyr-(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:10) and 510 mg (1.14 mmoles) 2-benzyl-5-di(p-methoxybenzyl)-amino valeric acid were coupled with 236 mg (1.14 mmoles) DCC, 177 mg (1.14 mmoles) 1-hydroxybenzotriazole, and 0.148 ml (1.14 mmoles) N-methylmorpholine as in Example 21. The product was purified by chromatography on silica gel using 0.1% NH_4OH/1% CH_3OH/CH_2Cl_2 to give 800 mg product, a 61% yield, as a 60:40 mixture of isomers. NMR ($CDCl_3$)δ: 6.5-7.4(m, 30H); 5.15(s, 2H); 5.01, 4.83(s, s 2H); 4.62(m, 1H); 4.1-4.5(m, 3H); 3.8, 3.78(s, s 6H); 3.26-3.56(m, 4H); 3.03-3.2(m, 2H); 2.3-2.9(m, 7H); 1-2(m, 14H); 0.8-1.0(m, 12H). MS m/e: 1114 (100%, M+1).

Step F: 2-Benzyl-5-aminopentanecarbonyl-Pro-Tyr-Ile-Leu (SEQ ID NO:88)

In a 35 ml flask 300 mg (0.28 mmoles) 2-benzyl-5-di(p-methoxybenzyl)aminopentanecarbonyl-Pro-Tyr-(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:89) was dissolved in 6 ml ethanol and 3 ml cyclohexene. In this 0.02 ml AcOH and 50 mg 20% $Pd(OH)_2/C$ was added and the mixture was heated to reflux for 11 hours. Then it was filtered through Celite®, stripped in vacuo, recrystallized from MeOH/EtOAc and chromatographed on silica gel using 20% $MeOH/CH_2Cl_2$, and 30% $MeOH/CH_2Cl_2$, to give 50 mg of isomer A, mp 161-163°C, and 40 mg isomer B, mp 166.2-168.2°C, as the neutral aminoacids, a 47% yield. A isomer (active): NMR ($CDCl_3$)δ: 7.0-7.42(m, 8H); 7.05(d, 2H, J=8HZ); 6.5(d, 2H, J=8HZ); 4.66(m, 1H); 4.15-4.38(m, 3H); 3.4(m, 1H); 3.1(m, 1H); 2.7-3.0(m, 7H); 1.0-1.9(m, 14H); 0.8-1.0(m, 12H). MS m/e: 694 (100%, M+1).

Example 36

4-t-Butoxycarbamidopiperidine-4-carbonyl)-
Pro-Tyr-Ile-Leu (SEQ ID NO:90)

Step A: 1-Benzyl-4-aminopiperidine-4-carboxylic acid

Benzyl-4-cyano-4-aminopiperidine, 3.0 g (13.95 mmoles), was dissolved in 30 ml of conc. HCl and heated at 120°C for 2 hours. Then 20 ml water was added and the pH was adjusted at 5-6 by addition of solid $NaHCO_3$. The product was filtered under reduced pressure as the hydrochloride salt and dried under vacuum to give 3.01 g of a white powder, an 80% yield.
NMR (D_2O): 7.4(s, 5H); 4.2(s, 2H); 3.4(m, 2H); 3.2-3.35(m, 2H); 2.2-2.3(m, 2H); 1.8-2.0(m, 2H). MS: 235 (M+1, 100%), 218, 189.

Anal. Calc'd. for $C_{13}H_{18}N_2O_2 \cdot HCl \cdot 1\text{-}1/2H_2O$:

	C 52.43	H 7.45	H 9.41
Found:	52.64	7.13	9.28.

Step B: 1-Benzyl-4-t-butoxycarbamidopiperidine-4-carboxylic acid

1-Benzyl-4-aminopiperidine-4-carboxylic acid, 2.0 g (7.4 mmoles), was dissolved in a mixture of 30 ml 0.5 M LiOH and 30 ml dioxane and was treated with 1.8 (10 mmoles) of $(Boc)_2O$ at 25°C for 16 hours and then with 3.6 g (20 mmoles) at 55°C for 11 hours. Then it was filtered at 25°C, neutralized with 10% $KHSO_4$, and the solvent was stripped off. The resulting solid was extracted with 100 ml hot methanol and filtered at 25°C. The solvent was removed and the white solid that remained was chromatographed on silica gel using 1:1 EtOAc/CH_3OH as eluent to give 1.1 g of product, a 45% yield. NMR (CD_3OD): 7.5(m, 2H); 7.4(m, 3H); 4.15(s, 2H); 3.2(m, 2H); 3.0(m, 2H); 2.25-2.4(m, 2H); 2.1-2.2(m, 2H).

Anal. Calc'd. for $C_{18}H_{26}N_2O_4 \cdot 2H_2O$:

	C 58.36	H 8.16	H 7.56
Found:	58.66	7.77	7.50.

Step C: (1-Benzyl-4-t-butoxycarbamidopiperidine-4-carbonyl)-Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:37)

Boc-Pro-Tyr(OBzl)-Ile-Leu(OBzl)•HCl (SEQ ID NO:9) 322 mg (0.5 mmoles), 167.5 mg (0.5 mmoles) 1-benzyl-4-t-butoxycarbamidopiperidine-4-carboxylic acid, 103 mg (0.5 mmoles) DCC and 76.5 mg (0.5 mmoles) 1-hydroxybenzotriazole hydrate were dissolved in 4 ml DMF at -10°C. To that 0.06 ml N-methylmorpholine was added and the reaction was continued at 25°C for 38 hours. The reaction was quenched with 50 ml 5% $NaHCO_3$ and extracted with 75 ml ethyl acetate. The EtOAc extracts were washed with brine, and evaporated to dryness in vacuo leaving a white solid. This was chromatographed on silica gel using 0.2% NH_4OH, 1% CH_3OH, CH_2Cl_2 as eluent to give 260 mg, a 58% of product, mp 196-198°C.

NMR ($CDCl_3$)δ: 7.2-7.4(m, 17H); 7.01-7.1(d, br s, 3H); 6.8-6.85(d, 2H); 5.15(s, 2H); 5.0(s br s, 3H); 4.6-4.75(m, 2H); 4.5(dd, 1H); 4.15-4.2(t, 1H); 3.75-3.8(m, 1H); 3.4-3.6(dd m, 3H); 2.6-3.0(m, 3H); 2.4-2.5(m, 1H); 2.05-2.3(m, 3H); 1.4-2.05(m, 13H); 1.35(s, 9H); 0.8-1.0(m, 12H).

MS: 1002(64%, M+2); 1001 (M+1, 100%), 911 (20%).

Anal. Calc'd.:	C 69.57	H 7.65	H 8.39
Found:	69.51	7.67	8.33.

Step D: (4-t-Butoxycarbamidopiperidine-4-carbonyl)-Pro-Tyr-Ile-Leu (SEQ ID NO:90)

In a 35 ml flask 270 mg (0.27 mmoles) (1-benzyl-4-t-butoxycarbamidopiperidine-4-carbonyl)-Pro-Tyr(OBzl)Ile-Leu(OBzl) (SEQ ID NO:37) was dissolved in 10 ml ethanol, 5 ml cyclohexene and 15 µl AcOH. To that 27 mg 20% $Pd(OH)_2/C$ was added and the mixture was heated to reflux for 4 hours under vigorous stirring. Then the solvent was stripped in vacuo and the resulting solid was dissolved in 400 ml 1:1 dioxane/water, filtered and stripped in vacuo. The product was precipitated from methanol to give 100 mg of a white solid, 60% yield, mp 227-228°C.

Example 37

N^{α}-(1-Adamantanecarbonyl)-Lys-(1-amino-1-cyclopentanecarbonyl)-Pro-Tyr-Ile-Leu (SEQ ID NO:96)

Step A: 1-t-Butoxycarbamido-1-cyclopentanecarboxylic acid

1-amino-1-cyclopentanecarboxylic acid, 2 g (15.49 mmoles), was dissolved in a mixture of 32 ml 0.5 M NaOH and 32 ml water and 3.75 g (17.04 mmoles) $(Boc)_2O$ was added. The mixture was stirred at 25°C for 2 hours, acidified with 10% $KHSO_4$ and extracted with EtOAc (2x40 ml). The combined extracts were washed with brine, dried and the solvent was stripped in vacuo. The resulting solid was chromatographed on silica gel using 33% EtOAc/Hexanes as eluent to give 900 mg of product, a 25% yield.

NMR ($CDCl_3$)δ: 4.95(br s, 1H); 2.2-2.35(m, 2H); 1.85-2.0(m, 2H); 1.8(m, 4H); 1.4(s, 9H). MS: 174, 156, 128 (100%).

Step B: (1-t-Butoxycarbamido-1-cyclopentanecarbonyl)-Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:97)

Pro-Tyr(OBzl)-Ile-Leu(OBzl)•HCl (SEQ ID NO:10) 910 mg (1.26 mmoles), 289 mg (1.26 mmoles), 1-t-butoxycarbamido-1-cyclopentanecarboxylic acid 260 mg (1.26 mmoles), 1,3 dicyclohexylcarbodiimide and 193 mg (1.26 mmoles) 1-hydroxybenzotriazole hydrate were dissolved in 4 ml DMF at -10°C. To that 0.06 ml N-methylmorpholine was added and the reaction was continued at 25°C for 48 hours. The reaction mixture was dissolved in 100 ml EtOAc, and washed with 5% $NaHCO_3$, water, 10% HCl, water and brine, 20 ml each time, dried and stripped under vacuum to give a solid. This was chromatographed on silica gel (30 g) using 5% CH_3OH/CH_2Cl_2 as eluent to give 530 mg, a 48% yield, of the product, mp 186-188°C. NMR ($CDCl_3$)δ: 7.2-7.5(m, 12H); 7.0-8.2(d, br s, 3H); 6.8-6.9(d, 2H); 5.18(s, 2H); 5.0(s, br s, 3H); 4.5-4.8(m, 3H); 4.25-4.35(t, 1H); 3.7-3.8(m, 1H); 3.4-3.55(m, 2H); 2.85-2.95(dd, 1H); 2.7-2.8(m, 1H); 2.1-2.3(m, 2H); 1.9-2.0(m, 1H); 1.4-1.9(m, 14H); 1.35(s, 9H); 0.8-1.0(m, 12H). MS: 914 (M+2, 60%); 913 (M+1, 100%).

Anal. Calc'd.:	C 68.35	H 7.76	H 7.82
Found:	68.32	7.75	7.71.

Step C: (1-amino-1-cyclopentanecarbonyl)-Pro-Tyr(OBzl)Ile-Leu(OBzl)•HCl (SEQ ID NO:93)

(1-t-Butoxycarbamido-1-cyclopentanecarbonyl)-Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:97) 450 mg (0.5 mmoles), was dissolved in 3 ml 4.5 M HCl in dioxane and stirred at 25°C for 3 hours. Then ether was added, and the precipitating salt was filtered under vacuum, to give 260 mg of product, a 62% yield. The product was used without further purification.

Step D: α-Boc-ε-CBZ-Lys-(1-amino-1-cyclopentane carbonyl)-Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:92)

1,3 Dicyclohexylcarbodiimide 64 mg (0.31 mmoles), α-Boc-ε-CBZ-Lys 118 mg (0.31 mmoles), (1-amino-1-cyclopentanecarbonyl)-Pro-Tyr(OBzl)-Ile-Leu(OBzl)•HCl (SEQ ID NO:93) 260 mg (0.31 mmoles) and 1-hydroxybenzotriazole hydrate 48 mg (0.33 mmoles) were dissolved in 2.5 ml DMF at -10°C. To that 0.040 ml (0.33 mmoles) N-methylmorpholine was added and the reaction was continued at 25°C for 48 hours. The reaction was quenched with 50 ml 5% $NaHCO_3$ and extracted with 75 ml ethyl acetate. The EtOAc extracts were washed with 10% HCl, 5% $NaHCO_3$ and brine, dried, and evaporated to dryness _in vacuo_ leaving a white solid. This was chromatographed on silica gel using 1% CH_3OH/CH_2Cl_2, to give 200 mg, a 55% yield of product.

NMR ($CDCl_3$)δ: 7.6(d, 1H); 7.0-7.4(m, 20H); 6.9(d, 2H); 5.95(br s, 1H); 5.2(br s, 1H); 5.15(s, 2H); 5.0-5.2(dd, 2H); 5.0(s, 2H); 4.45-4.65(m, 3H); 4.2-4.3(t, 1H); 4.0-4.1(m, 1H); 2.9-3.65(m, 7H); 2.7-2.8(m, 1H); 1.3-2.2(m, 22H); 1.45(s, 9H); 0.8-1.0(m, 12H).

MS m/e: 1175 (50%, $M+NH_4$); 1058 (50%, $M+H-C_4H_8-CO_2$).

Step E: ε-CBZ-Lys-(1-amino-1-cyclopentanecarbonyl)-Pro-Tyr(OBzl)-Ile-Leu(OBzl)•HCl (SEQ ID NO:95)

α-Boc-ε-CBZ-Lys-(1-amino-1-cyclopentanecarbonyl)-Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:92) 420 mg (0.36 mmoles) was dissolved in 1 ml 4.5 M HCl in dioxane and 2 ml CH_2Cl_2 and stirred at 25°C for 1 hour. Then ether was added and the product was precipitated as a white solid 350 mg, an 89% yield. This was used for the next reaction without further purification.

Step F: α-(1-Adamantanecarbonyl)-ε-CBZ-Lys-(1-amino-1-cyclopentanecarbonyl)-Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:91)

ε-CBZ-Lys-(1-amino-1-cyclopentanecarbonyl)-Pro-Tyr(OBzl)-Ile-Leu(OBzl)•HCl (SEQ ID NO:95) 350 mg (0.32 mmoles) which was dissolved in 5 ml CH_2Cl_2 and cooled to 0°C. To that 72 mg (0.35 mmoles) adamantanecarbonyl chloride was added following by 0.086 ml (0.70 mmoles) N-methylmorpholine. The mixture was stirred at 25°C for 16 hours and then poured into a separatory funnel containing 100 ml EtOAc and 20 ml $NaHCO_3$. The organic layer was washed with 20 ml 10% HCl, water and brine, dried and the solvent was stripped in vacuo. The remaining solid was purified by chromatography on silica gel using 4% MeOH/CH_2Cl_2 as eluent to give 320 mg of product, an 81% yield. MS m/e: 1238 (100%, M+NH_4).

Step G: α-(1-Adamantanecarbonyl)-Lys-(1-amino-1-cyclopentanecarbonyl)-Pro-Tyr-Ile-Leu (SEQ ID NO:96)

α-(1-Adamantanecarbonyl)-ε-CBZ-Lys-(1-amino-1-cyclopentanecarbonyl)-Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:91) 210 mg (0.17 mmoles) was dissolved in a mixture of 8 ml EtOH and 4 ml cyclohexene containing 10% w/w of 20% $Pd(OH)_2/C$ on carbon and 0.015 ml acetic acid. The mixture was heated to reflux for 4 hours, then filtered through Celite® and stripped in vacuo. The product was recrystallized from methanol/EtOAc to give 70 mg, a 42% yield mp 213-214°C. MS m/e: 907 (100%, M+).

Example 38

α-Boc-Lys-(1-amino-1-cyclopropanecarbonyl)-

Pro-Tyr-Ile-Leu (SEQ ID NO:99)

The compound was synthesized as described above in Example 37, mp 178.5-179.6°C.

Example 39

N^{α}(Boc)-Orn-Pro-Tyrψ[CH=CH]-Ile-Leu

(SEQ ID NO:56)

This compound can be prepared according to the procedure described above in Example 34.

Example 40

N^{α}(Boc)-Orn-Proψ[CH=CH]Tyr-Ile-Leu

(SEQ ID NO:100)

This compound can be prepared according to the procedure described above in Example 34.

Example 41

α-Boc-Lys-(1-amino-1-cyclopentane

carbonyl)-Pro-Tyr-Ile-Leu (SEQ ID NO:94)

α-Boc-ε-CBZ-Lys-(1-amino-1-cyclopentanecarbonyl)-Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:92) 190 mg (0.164 mmoles) was dissolved in a mixture of 6 ml EtOH and 3 ml cyclohexene containing 20 mg of 20% $Pd(OH)_2/C$ on carbon and 0.018 ml acetic acid. The mixture was heated to reflux for 4 hours, then filtered through Celite® and stripped <u>in vacuo</u>. The product was crystallized from methanol, mp 166-167°C.

Example 42

4-(1'-Adamantaneoxy)carbamidopiperidine-4-carbonyl-Pro-Tyr-Ile-Leu•AcOH (SEQ ID NO:98)

Step A: (1-Benzyl-4-(1'-adamantaneoxy)carbamidopiperidine-4-carboxylic acid

1-Benzyl-4-amino-4-piperidine-carboxylic acid hydrochloride hydrate 0.5 g (1.68 mmoles) was dissolved in a mixture of 3.5 ml 1M LiOH and 3.5 ml water. In this solution 7 ml dioxane and 333 mg (1.68 mmoles) 1-adamantyl fluoroformate was added and the resulting mixture was stirred at 25°C for 16 hours. The precipitated white solid was filtered off and dried, and used for the next reaction without purification.

Step B: (1-Benzyl-4-(1'-adamantaneoxy)carbamidopiperidine-4-carbonyl-Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:101)

(1-Benzyl-4-(1'-adamantaneoxy)carbamidopiperidine-4-carboxylic acid crude 300 mg (0.71 mmoles), HCl•Pro-Tyr(OBzl)-Ile-Leu(OBzl) (SEQ ID NO:10) 500 mg (0.69 mmoles), DCC 142.5 mg (0.69 mmoles), and 1-hydroxybenzotriazole 108 mg (0.69 mmoles) were dissolved in 6 ml DMF and cooled to -10°C. To that 0.09 ml (0.69 mmoles) N-methylmorpholine was added and the reaction was stirred at 25°C for 72 hours. Then it was quenched with $NaHCO_3$ (30 ml), and extracted with EtOAc (2x100 ml). The EtOAc was dried and stripped in vacuo. The resulting solid was chromatographed on silica gel using 0.25% NH_4OH/2.5% MeOH/CH_2Cl_2 as eluent to give 540 mg of product, a 72% yield, mp 79.5-81.5°C.

Anal. Calc'd for $C_{64}H_{82}N_6O_9$•1/2 H_2O:

C 70.62	H 7.68	N 7.72
70.52	7.67	7.77.

Step C: 4-(1'-adamantaneoxy)carbamidopiperidine-4-carbonyl-Pro-Tyr-Ile-Leu•AcOH (SEQ ID NO:98)

(1-Benzyl-4-(1'-adamantaneoxy)carbamidopiperidine-4-carbonyl-Pro-Tyr(OBzl)-Ile-Leu(OBzl) 300 mg (0.28 mmoles) was dissolved in 5 ml cyclohexene, 10 ml EtOH and 0.018 ml acetic acid. To that 50 mg 20% $Pd(OH)_2/C$ was added and the reaction was heated to reflux for 8 hours. Then the solvent was stripped *in vacuo* and the remaining solid was dissolved in 200 ml of 1:1 mixture hot water and dioxane. The solution was filtered through Celite® and stripped *in vacuo* to give 60 mg of product after precipitation from methanol, a 25% yield mp 261.7-262.2°C.

Neurotensin Binding Assay

Brain membrane preparations were prepared according to the method described in Tam (Proc. Natl. Acad. Sci. USA, **1983**, 80: 6703-6707). Whole brains (minus brainstem and cerebellum) were homogenized in ice-cold 0.34 M sucrose with a Brinkman Polytron (setting 8) for 20 seconds. The homogenate was centrifuged at 920 x g for 10 minutes. The supernatant was centrifuged at 47,000 x g for 20 minutes. The resulting membrane pellet was resuspended in 10 volumes (original wt/vol) of 50 mM Tris-HCl (pH 7.4) and incubated at 37°C for 45 minutes to degrade and dissociate bound endogenous ligands. The membranes were then centrifuged at 47,000 x g for 20 minutes and resuspended at a concentration of 2 brains per 90 ml of buffer, containing 50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 0.1% bovine serum albumin, and 50 mg/ml bacitracin.

Neurotensin receptor binding was performed according to the method of Goedert et al. (Brain Research, **1984**, 304: 71-81). One ml of the brain

membrane suspension containing 3 n$\underline{M}$ [^{3}H]neurotensin with or without test compounds was incubated at 25°C for 20 minutes. Nonspecific binding was defined by binding in the presence of 1 m$\underline{M}$ neurotensin. At the end of the incubation, the tubes were incubated at 4°C for 10 minutes and filtered rapidly under reduced pressure through Whatman GF/B glass fiber filters which have been pretreated for 3 hours with 0.2% polyethyleneimine in water. Each sample was washed 3 times with 5 ml ice cold incubation buffer. Radioactivity was determined by liquid scintillation spectrometry. IC_{50}s were calculated from log-logit plots. Apparent K_is were calculated from the equation $K_i = IC_{50}/[1+(L/K_d)]$ (Cheng and Prusoff, Biochem. Pharmacol., **1973**, 22, 3099), where L is the concentration of radioligand and K_d is its dissociation constant.

Analgesia Testing Procedure

The standard procedure for detecting and comparing the analgesic activity of compounds in this series is the phenylquinone writhing test (PQW) modified from E. Seigmund, et al., Proc. Soc. Exp. Biol. Med., **1957**, 95, 729. Intracerebroventricular (i.c.v.) injections were made according to the method of T. J. Haley and W. G. McCormick, Br. J. Pharmacol., **1957**, 12, 12-15.

Test compounds for i.v. administration were suspended in an aqueous vehicle containing 2% by volume of Tween® 80, a pharmacological dispersant manufactured by Fisher-Scientific Company and containing 100% polysorbate 80 and 0.25% by weight of Methocel® A15 powder, a suspending agent manufactured by Dow Chemical Company and containing 100% methylcellulose. I.V. doses were administered in a volume of 10 ml/kg body weight and are expressed as mg/kg doses. For i.c.v. administration, compounds were dissolved in 100%

dimethylsulfoxide (DMSO). Unilateral i.c.v. doses were injected in a volume of 5 microliters per mouse and are expressed as microgram/mouse doses. Test compounds were administered i.c.v. or i.v. to fasted (17-21 hours) male white mice (CF1), 5-15 animals per graded dose. After 5-25 minutes, aqueous 0.01% phenyl-p-benzoquinone, 0.125 mg/kg, was injected intraperitoneally. After an additional 5 minutes, mice were observed 10 minutes for the characteristic, stretching or writhing syndrome which is indicative of pain produced by phenylquinone. The effective analgesic dose in 50% of the mice (ED_{50}) was calculated by the moving average method of W. R. Thompson, Bac. Rev., **1947**, 11, 115-145. The mouse analgesic data are summarized in Table 1.

Ex. No.		Binding K_i (nM)	PQW Analgesia icv (µg)	iv(mg/kg)
1		144	1.7	14
2		3	0.68	13
3		117	0.15	2.2
4		1009	0.025	21
5		2059	3.1	16
6		540	0.057	12
7		1607	4.5	20
8		177	0.13	5.2
9		229	0.25	1.6
10		118	0.27	5.8
11		>10,000	1.3	6.6
12		598	0.027	3.3
13		419	0.0017	3.6
14		2985	0.89	37
15		>10,000	29	NT[1]
16		101	0.0095	5.5
17		193	0.29	16
18		676	0.08	4.7
19		884	1.6	13
20		217	0.07	7.5
22		>10,000	36	NT
23		3857	NT	NT
24		5959	NT	NT
25	SEQ ID NO:66	1340	2.4	NT
	SEQ ID NO:67	811	4.5	NT
	SEQ ID NO:68	270	0.89	6.5
	SEQ ID NO:69	511	NT	NT
	SEQ ID NO:70	9	0.18	0.32
	SEQ ID NO:71	182	0.15	6
	SEQ ID NO:72	154	1.7	19
	SEQ ID NO:73	80	4.5	NT
	SEQ ID NO:74	100	0.52	>9.0
	SEQ ID NO:75	257	0.47	0.50
	SEQ ID NO:76	310	1.3	>9.0
	SEQ ID NO:77	24	<50	NT
	SEQ ID NO:38	7	0.11	2.4
26		369	0.50	21
27		1263	1.6	>81
28		70	0.019	2.4
29		127	0.18	2.2
30		2363	0.18	14
31		2043	0.76	17
32		4848	NT	NT
33		5156	NT	NT

Ex.	Binding	PQW Analgesia	
No.	K_i (nM)	icv (µg)	iv(mg/kg)
34	1626	22	>27
35	735	0.0006	4.6
38	5960	NT	NT
41	6161	5.3	19
42	5803	2.0	27

[1] NT=Not Tested

Dosage Forms

The compounds of this invention may be administered by any means that produces contact of the active agent with the agent's site of action in the body of a mammal. They can be administered by any conventional means available for use in conjunction with pharmaceuticals, either as individual therapeutic agents or in a combination of therapeutic agents. They can be administered alone, but are generally administered with a pharmaceutical carrier selected on the basis of the chosen route of administration and standard pharmaceutical practice.

The dosage administered will, of course, vary depending upon known factors such as the pharmacodynamic characteristics of the particular agent, and its mode and route of administration; age, health, and weight of the recipient; nature and extent of symptoms, kind of concurrent treatment, frequency of treatment, and the effect desired. Usually a daily dosage of active ingredient can be about 0.1 to 100 milligrams per kilogram of body weight. Ordinarily 0.5 to 50, and preferably 1 to 10 milligrams per kilogram per day given in divided doses 1 to 6 times a day or in sustained release form is effective to obtain desired results.

Dosage forms (compositions) suitable for internal administration contain from about 1 milligram to about

500 milligrams of active ingredient per unit. In these pharmaceutical compositions the active ingredient will ordinarily be present in an amount of about 0.5-95% by weight based on the total weight of the composition.

The active ingredient can be administered orally in solid dosage forms, such as capsules, tablets, and powders, or in liquid dosage forms, such as elixirs, syrups, and suspensions. It can also be administered parenterally, in sterile liquid dosage forms, by inhalation in the form of a nasal spray or lung inhaler, or topically as an ointment, cream or lotion.

Gelatin capsules contain the active ingredient and powdered carriers, such as lactose, sucrose, mannitol, starch, cellulose derivatives, magnesium stearate, stearic acid, and the like. Similar diluents can be used to make compressed tablets. Both tablets and capsules can be manufactured as sustained release products to provide for continuous release of medication over a period of hours. Compressed tablets can be sugar coated or film coated to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric coated for selective disintegration in the gastrointestinal tract.

Liquid dosage forms for oral administration can contain coloring and flavoring to increase patient acceptance.

In general, water, a suitable oil, saline, aqueous dextrose (glucose), and related sugar solutions and glycols such as propylene glycol or polyethylene glycols are suitable carriers for parenteral solutions. Solutions for parenteral administration contain the active ingredient, suitable stabilizing agents, and if necessary, buffer substances. Antioxidizing agents such as sodium bisulfite, sodium sulfite, or ascorbic acid either alone or combined are suitable stabilizing

agents. Also used are citric acid and its salt and sodium EDTA. In addition, parenteral solutions can contain preservatives, such as benzalkonium chloride, methyl- or propyl-paraben, and chlorobutanol.

Suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences, A. Osol, a standard reference text in this field.

Useful pharmaceutical dosage forms for administration of the compounds of this invention can be illustrated as follows:

Capsules

A large number of unit capsules are prepared by filling standard two-piece hand gelatin capsules each with 50 milligrams of powdered active ingredient, 175 milligrams of lactose, 24 milligrams of talc, and 6 milligrams of magnesium stearate.

Soft Gelatin Capsules

A mixture of active ingredient in soybean oil is prepared and injected by means of a positive displacement pump into gelatin to form soft gelatin capsules containing 50 milligrams of the active ingredient. The capsules are washed in petroleum ether and dried.

Tablets

A large number of tablets are prepared by conventional procedures so that the dosage unit is 50 milligrams of active ingredient, 6 milligrams of magnesium stearate, 70 milligrams of microcrystalline cellulose, 11 milligrams of cornstarch and 225 milligrams of lactose. Appropriate coatings may be applied to increase palatability or delay absorption.

Injectable

A parenteral composition suitable for administration by injection is prepared by stirring 1.5% by weight of active ingredient in 10% by volume

propylene glycol and water. The solution is sterilized by commonly used techniques.

Suspension

An aqueous suspension is prepared for oral administration so that each 5 milliliters contain 25 milligrams of finely divided active ingredient, 200 milligrams of sodium carboxymethyl cellulose, 5 milligrams of sodium benzoate, 1.0 grams of sorbitol solution, U.S.P., and 0.025 milliliters of vanillin.

Nasal Spray

An aqueous solution is prepared such that each 1 milliliter contains 10 milligrams of active ingredient, 1.8 milligrams methylparaben, 0.2 milligrams propylparaben and 10 milligrams methylcellulose. The solution is dispensed into 1 milliliter vials.

Lung Inhaler

A homogeneous mixture of the active ingredient in polysorbate 80 is prepared such that the final concentration of the active ingredient will be 10 milligrams per container and the final concentration of polysorbate 80 in the container will be 1% by weight. The mixture is dispensed into each can, the valves are crimped onto the can and the required amount of dichlorotetrafluoroethane is added under pressure.

Topical Formulation

An ointment for topical administration may be prepared by adding the active ingredient to a mixture of 48% by weight white petrolatum, 10% liquid petrolatum, 8% glycerol monostearate, 3% isopropyl myristate and 20% lanolin at 70°C. After thorough mixing, a warm solution of methyl and propyl parabens in water containing sodium acetone bisulfite is added such that the final concentrations of each paraben is 0.15%, of water is 8% and of sodium acetone bisulfite is 0.5%. The mixture is stirred until it has reached room temperature.

CLAIMS

What is claimed is:

1. A compound of the formula

$$Y-(W)_m-(X)_n-A'-H-A-B-C-D-E-F-Z$$

wherein

Y is a lipophilic moiety having the structure L-C(O)-, or $R-(CH_2)_p-C(O)-(CH_2)_r-$, provided that when Y is L-C(O)- then L is selected from the group consisting of (i) at least one alkyl group having 1-16 carbon atoms, said alkyl group can be branched or unbranched, unsubstituted or substituted with at least one cyclic moiety selected from the group consisting of a cycloalkyl group having 3-8 carbon atoms, a heterocyclic group having 5-7 atoms in which the heteroatom is N, O, or S, or an aryl group having 6-15 carbon atoms wherein said aryl group can be unsubstituted or substituted with at least one alkyl group having 1-4 carbon atoms, (ii) perfluoroalkyl having 1-10 carbon atoms which can be unsubstituted or substituted with at least one cyclic group selected from the group consisting of an aryl group having 6-10 carbon atoms, a cycloalkyl group having 3-8 carbon atoms, or a heterocyclic group having 5-7 atoms in which the heteroatom is N, O, or S, (iii) cycloalkyl having 3-8 carbon atoms, (iv) bicycloalkyl having 6-18 carbon atoms, (v) tricycloalkyl having 6-18 carbon atoms, (vi) R^1-NH-R^2 wherein R^1 is H or alkyl having 1-4 carbon atoms; R^2 is selected from the group consisting of alkanediyl, branched or unbranched, having 1-16 carbon atoms, unsubstituted or substituted with at least one cyclic group selected from the group consisting of cycloalkyl having 3-8 carbon atoms, heterocyclic having 5-7 atoms in which the heteroatom is

N, O, or S, or an aryl group having 6-15 carbon atoms unsubstituted or substituted with at least one alkyl group having 1-4 carbon atoms, alkylcycloalkyl branched or unbranched having 4-16 carbon atoms wherein the cycloalkyl group has 3-8 carbon atoms, cycloalkylalkyl branched or unbranched having 4-16 carbon atoms wherein the cycloalkyl group has 3-8 carbon atoms, alkylaryl substituted with at least one moiety selected from the group consisting of alkyl, branched or unbranched, having 7-16 carbon atoms, said alkyl group being unsubstituted or substituted with NHR^1 or OH, said aryl group being unsubstituted or substituted with at least one alkyl group having 1-4 carbon atoms, arylalkyl substituted with at least one moiety selected from the group consisting of alkyl, branched or unbranched, having 7-16 carbon atoms, said alkyl group being unsubstituted or substituted with NHR^1 or OH, said aryl group being unsubstituted or substituted with at least one alkyl group having 1-4 carbon atoms, or alkylheterocyclic substituted with an alkyl group, branched or unbranched, having 6-16 carbon atoms, said heterocyclic having 5-7 atoms in which the heteroatom is N, O, or S,

further provided that when Y is $R-(CH_2)_p-C(O)-(CH_2)_r-$ then R is a cyclic group selected from the group consisting of cycloalkyl having 3-8 carbon atoms, heterocyclic having 5-7 atoms in which the heteroatom is N, O, S, or heterocyclic having 5-7 atoms in which the heteroatom is N and said heterocycle has at least one carbonyl moiety adjacent to the heteroatom, or aryl having 6-15 carbon atoms unsubstituted or substituted with at least one alkyl group having 1-4 carbon atoms; p and r are independently integers from 0 to 6;

W is an amino acid residue selected from the group consisting of arginine, lysine, ornithine, homoarginine,

2,4-diaminobutyric acid, 2,3-diaminopropionic acid, norleucine, N-methylnorleucine, D-arginine, D-lysine, proline, and 4-aminocyclohexylalanine.

X is an amino acid residue selected from the group consisting of arginine, lysine, ornithine, homoarginine, 2,4-diaminobutyric acid, 2,3-diaminopropionic acid, norleucine, N-methylnorleucine, D-arginine, D-lysine, proline, 4-aminocyclohexylalanine, alanine, or an alpha-amino acid residue substituted at the alpha carbon with at least one alkyl group having 1-6 carbon atoms, or said alpha-carbon atom is part of a cyclic moiety selected from the group consisting of cycloalkyl having 3-8 carbon atoms or heterocyclic having 3-8 atoms in which the heteroatom is N, O, or S;

m and n are independently 0 or 1, provided that m and n are not both 0 unless L is R^1-NH-R^2;

A', A, C, and E are independently selected from the group consisting of -CONH-, -CON(CH_3)-, -N(CH_3)CO-, -NHCR'R"-, -CR'R"NH-, -SO_2NR'R"-, -NR'R"SO_2-, -CH_2NH-, -CH_2O-, -CH_2S-, -NHCH_2-, -OCH_2-, -CSNH-, -NHCONH-, -S(O)CH_2-, -S(O)$_2$$CH_2$-, -NHSC-, -$CH_2$S(O)-, -$CH_2$S(O)$_2$-, -S$CH_2$-, cis- or trans- -CH=CH-, -NHCO-, -CH_2CH_2-, -CF_2CF_2-, -CF=CF-, -CF=CH-, -CH=CF-, -COCH_2-, -CH_2CO-, -CH(OH)CH_2-, -CH_2CH(OH)-, 1,2-cyclopropyldiyl, and 4,5-tetrazolyldiyl, wherein R' and R'' are independently lower alkyl groups having 1-6 carbon atoms;

H is an amino acid residue selected from the group consisting of proline or N-methylaminobutyric acid;

B is an amino acid residue selected from the group consisting of tyrosine, phenylalanine, tryptophan, naphthylalanine, phenylglycine, and beta-phenylproline;

D is an amino acid residue selected from the group consisting of isoleucine, leucine, tert-leucine, and phenylglycine;

F is an amino acid residue selected from the group consisting of leucine, valine, and methionine; and

Z is OH or OR^3 wherein R^3 is an alkyl group having 1-6 carbon atoms.

2. A compound according to claim 1 wherein Y is a lipophilic moiety having the structure L-C(O)- or R-$(CH_2)_p$-C(O)-$(CH_2)_r$-, provided that when Y is L-C(O)- then L is selected from the group consisting of (i) alkyl, branched or unbranched, having 1-16 carbon atoms, (ii) perfluoroalkyl having 1-10 carbon atoms, (iii) cycloalkyl having 3-8 carbon atoms, (iv) bicycloalkyl having 6-18 carbon atoms, (v) tricycloalkyl having 6-18 carbon atoms, (vi) R^1-NH-R^2- wherein R^1 is H or alkyl having 1-4 carbon atoms, R^2 is selected from the group consisting of alkanediyl, branched or unbranched having 1-16 carbon atoms, alkylaryl substituted with at least one moiety selected from the group consisting of alkyl, branched or unbranched, having 7-16 carbon atoms, said alkyl group being unsubstituted or substituted with NHR^1 or OH, said aryl group being unsubstituted or substituted with at least alkyl group having 1-4 carbon atoms, or arylalkyl substituted with at least one moiety selected from the group consisting of alkyl, branched or unbranched, having 7-16 carbon atoms, said alkyl group being unsubstituted or substituted with NHR^1 or OH, said aryl group being unsubstituted or substituted with at least one alkyl group having 1-4 carbon atoms;

further provided that when Y is R-$(CH_2)_p$-C(O)-$(CH_2)_r$- then R is a cyclic group selected from the group consisting of cycloalkyl having 3-8 carbon atoms, aryl having 6-15 carbon atoms unsubstituted or substituted with at least one alkyl group having 1-4 carbon atoms, heterocyclic having 5-7 atoms in which the heteroatom is N, O, or S, or heterocyclic having 5-7 atoms in which the heteroatom is N and said heterocycle has at least

one carbonyl moiety adjacent to the heteroatom; p and r are independently integers from 0 to 6;

W is an amino acid residue selected from the group consisting of arginine, lysine, ornithine, 2,4-diaminobutyric acid, norleucine, N-methylnorleucine, D-arginine, 4-aminocyclohexylalanine, or proline;

X is an amino acid residue selected from the group consisting of arginine, lysine, ornithine, 2,4-diaminobutyric acid, norleucine, N-methylnorleucine, D-arginine, proline, 4-aminocyclohexylalanine, alanine, or an alpha-amino acid residue in which the alpha carbon is part of cyclic moiety selected from the group consisting of cycloalkyl having 3-8 carbon atoms or heterocyclic having 3-8 atoms in which the heteroatom is N, O, or S;

m and n are independently 0 or 1, provided that m and n are not both 0 unless L is R^1-NH-R^2-;

A', A, C, and E are independently selected from the group consisting of -CONH-, -CH_2NH-, -CH_2O-, -CH_2S-, -NHCH_2-, -OCH_2-, -CSNH-, -NHSC-, -SCH_2-, cis- or trans- -CH=CH-, -NHCO-, -CH_2CH_2-, -CF_2CF_2-, -CF=CF-, -CF=CH-, -CH=CF-, -COCH_2-, -CH_2CO-, -CH(OH)CH_2-, -CH_2CH(OH)-;

H is an amino acid residue selected from the group consisting of proline or N-methylaminobutyric acid;

B is an amino acid residue selected from the group consisting of tyrosine, phenylalanine, tryptophan, naphthylalanine, phenylglycine, and beta-phenylproline;

D is an amino acid residue selected from the group consisting of isoleucine, leucine, tert-leucine, and phenylglycine;

F is an amino acid residue selected from the group consisting of leucine, valine, and methionine; and

Z is OH or OR^3 wherein R^3 is alkyl having 1-6 carbon atoms.

3. A compound according to claim 1 wherein

Y is selected from the group consisting of acetyl, pivaloyl, neopentylcarbonyl, n-perfluoroctanoyl, 1-bicyclo[3.3.0]octanecarbonyl, 2-bicyclo[2.2.1]heptane-acetyl, 1-adamantanecarbonyl, 2-pyrrolidinecarbonyl (prolyl), 2-(5-pyrrolid-5-one)-carbonyl[pyroglutamyl], benzoyl, 4-tert-butylbenzoyl, 4-phenylbenzoyl, nicotinoyl, 2-benzyl-5-aminopentanoyl, trans-4-(aminomethyl)-cyclohexanecarbonyl, 2-(aminomethyl)-benzoyl, and 4-(aminocyclohexyl)-alanyl;

W is an arginine residue;

X is an amino acid residue selected from the group consisting of arginine, lysine, ornithine, 4-aminocyclohexylalanine, 4-aminopiperidine-4-carboxylic acid, 1-aminocyclopentanecarboxylic acid, 1-aminocyclobutanecarboxylic acid, or 1-amino-cyclopropanecarboxylic acid;

m and n are independently 0 or 1, provided that m and n are not both zero, except when Y is 2-benzyl-5-aminopentanoyl then m and n can be zero, and further provided that when Y is acetyl then m and n are 1;

A', C, and E are -CONH-;

A is -CONH- or $-CH_2NH-$;

H is a proline residue;

B is an amino acid residue selected from the group consisting of tyrosine and tryptophan;

D is an amino acid residue selected from the group consisting of isoleucine, tert-leucine, and phenylglycine;

F is a leucine residue;

Z is OH or OCH_3.

4. A compound according to claim 1 wherein

Y is selected from the group consisting of 1-adamantanecarbonyl, 2-benzyl-5-aminopentanoyl, benzoyl, nicotinoyl, and acetyl;

W is an arginine residue;

X is an amino acid residue selected from the group consisting of arginine, lysine, and ornithine;

m and n are independently 0 or 1, provided that m and n are not both zero, except when Y is 1-benzyl-5-aminopentanoyl then m and n can be zero, and further provided that when Y is acetyl, both m and n are 1;

A', A, C, and E are -CONH-;

H is a proline residue;

B is an amino acid residue selected from the group consisting of tyrosine and tryptophan;

D is an amino acid residue selected from the group consisting of isoleucine, tert-leucine, and phenylglycine;

F is a leucine residue;

Z is OH or OCH_3.

5. A compound according to claim 1 wherein

Y is selected from the group consisting of 1-adamantanecarbonyl, 2-norbornaneacetyl, 1-perfluorooctanoyl;

W is an amino acid residue selected from the group consisting of Arg, Lys, Orn;

X is an amino acid residue selected from the group consisting of Arg, Lys, Orn, 1-aminocyclopentane-1-carbonyl, 2-, 3-, or 4-amino-piperidine-2-, 3-, or 4-carbonyl;

m and n are independently 0 or 1 provided that m and n are not both 0;

A, C, and E are independently -CO-NH-, $-CH_2NH-$, or trans-CH=CH;

B is an amino acid residue selected from the group consisting of Tyr, Phe, Trp;

D is amino acid residue selected from the group consisting of Ile, Leu, Pgl, Gly;

F is an amino acid residue selected from the group consisting of Leu, Val; and

Z is OH or OCH_3.

6. A compound according to claim 1 wherein

Y is 1-adamantanecarbonyl;

W and X are independently Arg or Lys;

m and n are independently 0 or 1 provided that m and n are not both 0;

A, C and E are independently -CONH-, $-CH_2NH-$, or trans-CH=CH-;

B is Tyr;

D is Ile;

F is Leu; and

Z is OH or OCH_3.

7. A compound according to claim 1 which is selected from the group consisting of:

N^{α}-(1-adamantanecarbonyl)-Arg-Pro-Tyr-Ile-Leu;

N^{α}-(1-adamantanecarbonyl)-Arg-Arg-Pro-Tyr-Ile-Leu;

N^{α}-(1-adamantanecarbonyl)-Lys-Pro-Tyr-Ile-Leu;

N^{α}-(1-adamantanecarbonyl)-Lys-Pro-Ψ[CH_2NH]-Tyr-Ile-Leu;

N^{α}-(1-adamantanecarbonyl)-Lys-Pro-Ψ[CH=CH]-Tyr-Ile-Leu;

N^{α}-(cis-bicyclo(3.3.0)octane-2-carbonyl)-Lys-Pro-Tyr-Ile-Leu;

N^{α}-(1-adamantanecarbonyl)-Orn-Pro-Tyr-Ile-Leu;

N^{α}-(1-adamantanecarbonyl)-Lys-Pro-Trp-Ile-Leu;

N^{α}-(1-adamantanecarbonyl)-Lys-Pro-Tyr-(S)-2-phenylglycyl-Leu;

N^{α}-(2-norbornaneacetyl)-Lys-Pro-Tyr-Ile-Leu;

N^{α}-($CF_3(CF_2)_6CO$)-Lys-Pro-Tyr-Ile-Leu;

4-(1'-adamantanecarbamido)-4-piperidine-carbonyl-Pro-Tyr-Ile-Leu;

N^{α}(1-adamantanecarbonyl)Lys-Pro-Tyr-Ile-Leu(OMe);

N^{α}(nicotinoyl)Lys-Pro-Tyr-Ile-Leu;

N^{α}(Boc)Orn-Pro-ψ[CH_2NH]Tyr-Ile-Leu;

N^{α}(Boc)Orn-Pro-Tyrψ[CH_2NH]-Ile-Leu;

N^{α}(Boc)Orn-Pro-Tyrψ[CH=CH]-Ile-Leu;

N^{α}(Boc)Orn-Pro-ψ[CH=CH]-Tyr-Ile-Leu;

N^{α}-(PhCO)-Lys-Pro-Tyr-Ile-Leu;
N^{α}(t-BuCO)-Lys-Pro-Tyr-Ile-Leu;
N^{α}-(t-BuCH$_2$CO)-Lys-Pro-Tyr-Ile-Leu;
N^{α}-(4-Ph-C$_6$H$_4$-CO)-Lys-Pro-Tyr-Ile-Leu;
N^{α}-(4-t-Bu-C$_6$H$_4$-CO)-Lys-Pro-Tyr-Ile-Leu;
N-(2-benzyl-5-aminopentanoyl)-Pro-Tyr-Ile-Leu;
N^{α}-(1-adamantanecarbonyl)-Arg-Arg-Pro-Tyr-Tle-Leu;
N^{α}-acetyl-Arg-Arg-Pro-Tyr-S-2-phenyglycyl-Leu; or
N^{α}-(1-adamantanecarbonyl)-Lys-Pro-Tyr-Tle-Leu.

8. A pharmaceutical composition comprising a suitable pharmaceutical carrier and an antipsychotic amount of a compound of claims 1-7.

9. A method of treating psychosis in a mammal which comprises administering to the mammal an antipsychotic effective amount of a compound of claims 1-7.

10. A method of treating pain in a mammal which comprises administering to the mammal an analgesic effective amount of a compound of claims 1-7.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 92/04968

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07K7/08; C07K7/02; C07K5/02; A61K37/02

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07K ; A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
A	EP,A,0 333 071 (EISAI CO.) 20 September 1989 cited in the application see the whole document ---	1-10
X	US,A,4 425 269 (CHRISTY ET AL.) 10 January 1984 cited in the application see examples 1-7 ---	1-10

-/--

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
07 OCTOBER 1992	02. 11. 92
International Searching Authority	Signature of Authorized Officer
EUROPEAN PATENT OFFICE	P. MASTURZO

2

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	BIOCHEMICAL PHARMACOLOGY vol. 36, no. 6, 1987, GB pages 869 - 874 K S KANBA ET AL. 'comparison of the stimulation of inositol phospholipid hydrolysis and of cGMP formation by neurotensin, some of its analogs and neuromedin N in neuroblastoma clone NIE-115' see table 1	1-10
P,X	203RD ACS NAT. MEETING, S. FRANCISCO, CALIFORNIA, APRIL 5-10,1992, ABSTRACT PAPERS: ABSTRACT NO. 84 vol. 203, no. 1-3, G A CAIN ET AL. 'neurotensin based analgesic identification of minimally active fragment : enhancement of potency, duration of action, and transport properties' see the whole document	1-10
P,X	203RD ACS NAT. MEETING, S. FRANCISCO, CALIFORNIA, APRIL 5-10, 1992, ABSTRACT PAPERS, ABSTRACT NO 81 vol. 203, no. 1-3, W K SCHMIDT ET AL. 'adamantoyl-lys-pro-tyr-ile-leu, ada-kypil, a systematically active neurotensin 9-13 analog with ana analgetic and antipsychotic profile in mice and rats' see the whole document	1-10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 92/04968

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 9-10 refers to a method of treatment of the human body, the search has been carried out and based on the alleged effects of the compounds

2. [X] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
see annex.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

In view of the extremely large number of compounds falling under claim 1 and 2, and of the absence of any sensible support for these claims in the description, the Search division considers that it is not economically reasonable to draw a search report covering the entire subject matter of claims 1,2 and dependant claims 8 to 10.
The search report has therefore been limited to claims 3 to 7, to claims 8- 10 as far as they are dependent from claims 3 to 7 and includes all the real examples given in the description.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9204968
SA 61818

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 07/10/92
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0333071	20-09-89	AU-A-	3108389	14-09-89
		JP-A-	1316399	21-12-89
US-A-4425269	10-01-84	None		

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82